

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

GILES, Allen, Frank
AstaZeneca UK Limited
Global Intellectual Property
Mereside, Alderley Park
Macclesfield
Cheshire SK10 4TG
ROYAUME-UNI

Date of mailing (day/month/year) 22 May 2000 (22.05.00)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference PHM 70311/WO	
International application No. PCT/GB99/01793	International filing date (day/month/year) 08 June 1999 (08.06.99)

1. The following indications appeared on record concerning:

☒ the applicant ☐ the inventor ☐ the agent ☐ the common representative

Name and Address ZENECA LIMITED 15 Stanhope Gate London W1Y 6LN United Kingdom	State of Nationality GB	State of Residence GB
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☒ the person ☐ the name ☐ the address ☐ the nationality ☐ the residence

Name and Address ASTRAZENECA UK LIMITED 15 Stanhope Gate London W1Y 6LN United Kingdom	State of Nationality GB	State of Residence GB
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office ☐ the designated Offices concerned
☐ the International Searching Authority ☒ the elected Offices concerned
☒ the International Preliminary Examining Authority ☐ other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer H. Zhou
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF THE RECORDING
OF A CHANGE(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

GILES, Allen, Frank
AstraZeneca
Global Intellectual Property
P.O. Box 272
Mereside, Alderley Park
Macclesfield, Cheshire SK10 4GR
ROYAUME-UNI

Date of mailing (day/month/year) 28 September 2000 (28.09.00)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference PHM 70311/WO	
International application No. PCT/GB99/01793	International filing date (day/month/year) 08 June 1999 (08.06.99)

1. The following indications appeared on record concerning:		
<input checked="" type="checkbox"/> the applicant	<input type="checkbox"/> the inventor	<input type="checkbox"/> the agent <input type="checkbox"/> the common representative
Name and Address ASTRAZENECA UK LIMITED 15 Stanhope Gate London W1Y 6LN United Kingdom	State of Nationality GB	State of Residence GB
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:		
<input checked="" type="checkbox"/> the person	<input checked="" type="checkbox"/> the name	<input checked="" type="checkbox"/> the address <input checked="" type="checkbox"/> the nationality <input checked="" type="checkbox"/> the residence
Name and Address ASTRAZENECA AB S-151 85 Södertälje Sweden	State of Nationality SE	State of Residence SE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	
3. Further observations, if necessary:		
4. A copy of this notification has been sent to:		
<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/> the designated Offices concerned	
<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned	
<input checked="" type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:	

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Athina Nickitas-Etienne Telephone No.: (41-22) 338.83.38
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To:

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Macclesfield, Cheshire SK10 4GR
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Applicant's or agent's file reference PHM 70311/WO	
International application No. PCT/GB99/01793	International filing date (day/month/year) 08 June 1999 (08.06.99)

1. The following indications appeared on record concerning:		
<input type="checkbox"/> the applicant	<input type="checkbox"/> the inventor	<input checked="" type="checkbox"/> the agent
<input type="checkbox"/> the common representative		
Name and Address GILES, Allen, Frank AstraZeneca UK Limited Global Intellectual Property Mereside, Alderley Park Macclesfield Cheshire SK10 4TG United Kingdom	State of Nationality	State of Residence
	Telephone No. (01625) 516573	
	Facsimile No. (01625) 583358	
	Teleprinter No.	
2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:		
<input type="checkbox"/> the person	<input type="checkbox"/> the name	<input checked="" type="checkbox"/> the address
<input type="checkbox"/> the nationality		
<input type="checkbox"/> the residence		
Name and Address GILES, Allen, Frank AstraZeneca Global Intellectual Property P.O. Box 272 Mereside, Alderley Park Macclesfield, Cheshire SK10 4GR United Kingdom	State of Nationality	State of Residence
	Telephone No. 01625 516573	
	Facsimile No. 01625 583358	
	Teleprinter No.	
3. Further observations, if necessary:		
4. A copy of this notification has been sent to:		
<input checked="" type="checkbox"/> the receiving Office	<input type="checkbox"/> the designated Offices concerned	
<input type="checkbox"/> the International Searching Authority	<input checked="" type="checkbox"/> the elected Offices concerned	
<input checked="" type="checkbox"/> the International Preliminary Examining Authority	<input type="checkbox"/> other:	

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Athina Nickitas-Etienne Telephone No.: (41-22) 338.83.38
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AstaZeneca UK Limited
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Macclesfield
Cheshire SK10 4TG
ROYAUME-UNI

Date of mailing (day/month/year) 22 May 2000 (22.05.00)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference PHM 70311/WO	
International application No. PCT/GB99/01793	International filing date (day/month/year) 08 June 1999 (08.06.99)

1. The following indications appeared on record concerning:

☐ the applicant ☐ the inventor ☒ the agent ☐ the common representative

Name and Address

GILES, Allen, Frank
AstaZeneca PLC
Global Intellectual Property
Mereseide, Alderley Park
Macclesfield
Cheshire SK10 4TG
United Kingdom

State of Nationality

State of Residence

Telephone No.

(01625) 516573

Facsimile No.

(01625) 583358

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address

GILES, Allen, Frank
AstaZeneca UK Limited
Global Intellectual Property
Mereseide, Alderley Park
Macclesfield
Cheshire SK10 4TG
United Kingdom

State of Nationality

State of Residence

Telephone No.

(01625) 516573

Facsimile No.

(01625) 583358

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office ☐ the designated Offices concerned
☐ the International Searching Authority ☒ the elected Offices concerned
☒ the International Preliminary Examining Authority ☐ other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

H. Zhou

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

NOTIFICATION OF ELECTION

(PCT Rule 61.2)

From the INTERNATIONAL BUREAU

To:

Assistant Commissioner for Patents
United States Patent and Trademark
Office
Box PCT
Washington, D.C.20231
ÉTATS-UNIS D'AMÉRIQUE

in its capacity as elected Office

Date of mailing (day/month/year) 25 January 2000 (25.01.00)	
International application No. PCT/GB99/01793	Applicant's or agent's file reference PHM 70311/WO
International filing date (day/month/year) 08 June 1999 (08.06.99)	Priority date (day/month/year) 12 June 1998 (12.06.98)
Applicant LU, Jian, Jason et al	

1. The designated Office is hereby notified of its election made:

☒ in the demand filed with the International Preliminary Examining Authority on:15 December 1999 (15.12.99)☐ in a notice effecting later election filed with the International Bureau on:2. The election ☒ was☐ was not

made before the expiration of 19 months from the priority date or, where Rule 32 applies, within the time limit under Rule 32.2(b).

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Olivia RANAIVOJAONA Telephone No.: (41-22) 338.83.38
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INTERNATIONAL SEARCH REPORT

International Application No.
PCT/GB 99/01793

A. CLASSIFICATION OF SUBJECT MATTER IPC 6 C12N15/12 C07K14/705 C07K16/28 A61K38/17		
According to International Patent Classification (IPC) or to both national classification and IPC		
B. FIELDS SEARCHED Minimum documentation searched (classification system followed by classification symbols) IPC 6 C12N C07K A61K		
Documentation searched other than minimum: documentation to the extent that such documents are included in the fields searched		
Electronic data base consulted during the international search (name of data base and, where practical, search terms used)		
C. DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	PAN G. ET AL.: "Identification and functional characterization of DR6, a novel death domain-containing TNF receptor" FEBS LETTERS, vol. 431, 1998, pages 351-356, XP002113408 the whole document	1-9
P,X	WO 99 15663 A (MILLENNIUM BIOTHERAPEUTICS INC) 1 April 1999 (1999-04-01) sequence of TRL II	1-9
P,X	EP 0 869 179 A (SMITHKLINE BEECHAM CORP) 7 October 1998 (1998-10-07) sequence of TR7	1-9
-/--		
<div style="display: flex; justify-content: space-between;"> <input checked="" type="checkbox"/> Further documents are listed in the continuation of box C. <input checked="" type="checkbox"/> Patent family members are listed in annex. </div>		
* Special categories of cited documents : <div style="display: flex; justify-content: space-between;"> <div style="width: 45%;"> <p>"A" document defining the general state of the art which is not considered to be of particular relevance</p> <p>"E" earlier document but published on or after the international filing date</p> <p>"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)</p> <p>"O" document referring to an oral disclosure, use, exhibition or other means</p> <p>"P" document published prior to the international filing date but later than the priority date claimed</p> </div> <div style="width: 45%;"> <p>"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention</p> <p>"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone</p> <p>"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.</p> <p>"&" document member of the same patent family</p> </div> </div>		
Date of the actual completion of the international search	Date of mailing of the international search report	
26 August 1999	06/09/1999	
Name and mailing address of the ISA European Patent Office, P.O. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040. Tx. 31 651 epo nl. Fax: (+31-70) 340-3016	Authorized officer Galli, I	

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/01793

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 56892 A (HUMAN GENOME SCIENCES INC ;NI JIAN (US); FAN PING (US); GENTZ REIN) 17 December 1998 (1998-12-17) sequence of TR9	1-9
P,X	WO 99 11790 A (ZYMOGENETICS INC) 11 March 1999 (1999-03-11) sequence of ZTNFR-6	1-9
E	WO 99 31128 A (INCYTE PHARMA INC ;AU YOUNG JANICE (US); BANDMAN OLGA (US); KASER) 24 June 1999 (1999-06-24) seq. ID 4	1-9
A	KITSON J ET AL: "A DEATH-DOMAIN-CONTAINING RECEPTOR THAT MEDIATES APOPTOSIS" NATURE, vol. 384, no. 6607, 28 November 1996 (1996-11-28), pages 372-375, XP002036560 ISSN: 0028-0836 abstract	1-9
A	CHINNAIYAN A M ET AL: "SIGNAL TRANSDUCTION BY DR3, A DEATH DOMAIN-CONTAINING RECEPTOR RELATED TO TNFR-1 AND CD95" SCIENCE, vol. 274, no. 5289, 8 November 1996 (1996-11-08), pages 990-992, XP000676685 ISSN: 0036-8075 abstract	1-9
A	PAN G ET AL: "AN ANTAGONIST DECOY RECEPTOR AND A DEATH DOMAIN-CONTAINING RECEPTOR FOR TRAIL" SCIENCE, vol. 277, 8 August 1997 (1997-08-08), pages 815-818, XP002065147 ISSN: 0036-8075 abstract	1-9
A	PAN G ET AL: "THE RECEPTOR FOR THE CYTOTOXIC LIGAND TRAIL" SCIENCE, vol. 276, 4 April 1997 (1997-04-04), pages 111-113, XP002913702 ISSN: 0036-8075 abstract	1-9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 99/01793

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 9 can be understood to comprise methods in vivo and is therefore considered a method of treatment of the human/animal body, the search has been limited to the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/01793

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9915663	A	01-04-1999	AU 9777698 A	12-04-1999
EP 0869179	A	07-10-1998	JP 11018786 A	26-01-1999
WO 9856892	A	17-12-1998	AU 7830398 A	30-12-1998
WO 9911790	A	11-03-1999	AU 9130598 A	22-03-1999
WO 9931128	A	24-06-1999	NONE	

PATENT COOPERATION TREATY

PCT

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

09 / 733 956

REC'D 28 SEP 2000

WIPO

PCT

15

Applicant's or agent's file reference PHM 70311/WO	FOR FURTHER ACTION See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)	
International application No. PCT/GB99/01793	International filing date (day/month/year) 08/06/1999	Priority date (day/month/year) 12/06/1998
International Patent Classification (IPC) or national classification and IPC C12N15/12		
Applicant ZENECA LIMITED et al.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.


2. This REPORT consists of a total of 7 sheets, including this cover sheet.

☐ This report is also accompanied by ANNEXES, i.e. sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

These annexes consist of a total of sheets.

3. This report contains indications relating to the following items:

- I ☒ Basis of the report
- II ☒ Priority
- III ☐ Non-establishment of opinion with regard to novelty, inventive step and industrial applicability
- IV ☐ Lack of unity of invention
- V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement
- VI ☒ Certain documents cited
- VII ☐ Certain defects in the international application
- VIII ☒ Certain observations on the international application

Date of submission of the demand 15/12/1999	Date of completion of this report 26.09.2000
Name and mailing address of the international preliminary examining authority:  European Patent Office D-80298 Munich Tel. +49 89 2399 - 0 Tx: 523656 epmu d Fax: +49 89 2399 - 4465	Authorized officer Julia, P Telephone No. +49 89 2399 8410



INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/01793

I. Basis of the report

1. This report has been drawn on the basis of *(substitute sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to the report since they do not contain amendments.)*:

Description, pages:

1-29 as originally filed

Claims, No.:

1-9 as originally filed

2. The amendments have resulted in the cancellation of:

- ☐ the description, pages:
- ☐ the claims, Nos.:
- ☐ the drawings, sheets:

3. ☐ This report has been established as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed (Rule 70.2(c)):

4. Additional observations, if necessary:

II. Priority

1. ☐ This report has been established as if no priority had been claimed due to the failure to furnish within the prescribed time limit the requested:
- ☐ copy of the earlier application whose priority has been claimed.
 - ☐ translation of the earlier application whose priority has been claimed.
2. ☐ This report has been established as if no priority had been claimed due to the fact that the priority claim has been found invalid.

Thus for the purposes of this report, the international filing date indicated above is considered to be the relevant date.

INTERNATIONAL PRELIMINARY EXAMINATION REPORT

International application No. PCT/GB99/01793

3. Additional observations, if necessary:

see separate sheet

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. Statement

Novelty (N)	Yes:	Claims	1-9
	No:	Claims	
Inventive step (IS)	Yes:	Claims	1-9
	No:	Claims	
Industrial applicability (IA)	Yes:	Claims	1-8
	No:	Claims	9 (see citations and explanations)

2. Citations and explanations

see separate sheet

VI. Certain documents cited

1. Certain published documents (Rule 70.10)

and / or

2. Non-written disclosures (Rule 70.9)

see separate sheet

VIII. Certain observations on the international application

The following observations on the clarity of the claims, description, and drawings or on the question whether the claims are fully supported by the description, are made:

see separate sheet

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

International application No. PCT/GB99/01793

1. Additional remarks to item I :

A "Sequence Listing" has been filed with the present application. This "Sequence Listing" comprises SEQ ID No.: 1 to SEQ ID No.: 2 (pages 1-4).

2. Additional remarks to item II :

The priority documents pertaining to the present application were not available at the time of establishing this international preliminary examination report (IPER). Hence, the current assessment is based on the assumption that all claims enjoy priority rights from the filing date of the priority document (12.06.98). If it later turns out that this is not correct, the document Pang et al., FEBS Letters 1998, Vol. 431, pages 351-356 cited in the International Search Report (ISR) could become relevant to assess whether the claimed subject matter of the present application satisfies the criteria set forth in Article 33 (1) PCT.

3. Additional remarks to item V :

The present application discloses the cDNA sequence (SEQ ID No.: 1) of clone g1727750 made from mRNA of a human colon epithelial cell line encoding a 45 kDa protein (SEQ ID No.: 2), named DETH for **D**eath **D**omain **E**xpressing **T**umour **N**ecrosis **F**actor **R**eceptor **F**amily **H**omologue, which is a member of the TNFR1/FAS/NGFR family of receptors and which is shown to have a role in apoptosis.

None of the documents published in time and cited in the International Search Report (ISR) discloses the specific sequence of the DETH protein or the corresponding nucleic acid. J. Kitson et al., Nature 1996, Vol. 384, pages 372-375 (**D1**) concerns the isolation of WSL-1, a member of the TNFR superfamily, by using the death domain of TNFR1 in a yeast-two hybrid system. The document shows a sequence comparison of the death domains of TRADD, TNFR1 and WSL-1. A.M. Chinnaiyan et al., Science 1996, Vol. 274, pages 990-992 (**D2**) also discloses the death receptor 3 (DR3), a member of the TNFR1 family isolated from tissues enriched in lymphocytes. The document shows a sequence comparison of said DR3 with TNFR-1 and FAS (APO-1 or CD95). The documents G. Pan et al., Science 1997, Vol. 277, pages 815-818 (**D3**) and G. Pan et al., Science 1997, Vol. 276, pages 111-113 (**D4**) are concerned with the death receptor 4 (DR4). The ISA has not found any significant homology among those TNFR-1 members and the DETH disclosed in the present application.

In view of this cited prior art, the IPEA considers that none of these documents anticipates

the claimed subject matter (Article 33 (2) PCT) and that there is no suggestion, motivation or indication in said prior art that could have led the skilled person to achieve the claimed subject matter in an obvious manner (Article 33 (3) PCT).

The attention of the Applicant is also drawn to the fact that the subject matter of claim 9, as far as directed to an in vivo method, can be seen as comprising a method for treatment of the human or animal body (insofar the claimed subject matter comprises embraces a method in vivo too) and thus, it may be excluded from examination by Article 34(4)(a)(i) PCT in combination with Rule 67(iv) PCT too. Furthermore, for such a subject matter no unified criteria exist in PCT for the assessment whether it is industrially applicable or not. The patentability can also be dependent upon the formulation of the claims. The EPO, for example, does not recognize as industrially applicable the subject matter of claims to the use of a compound in medical treatment, but will allow, however, claims to a known compound for first use in medical treatment and the use of such a compound for the manufacture of a medicament for a new medical treatment.

4. Additional remarks to item VI :

Certain published documents (**Rule 70.10 PCT**) : WO-A-99/15663, publication date: 01.04.99, priority dates: 26.09.97 and 17.03.98, filing date: 25.09.98; EP-A-0 869 179, publication date: 07.10.98, priority dates: 02.04.97 and 28.10.97, filing date: 01.04.98; WO-A-98/56892, publication date: 17.12.98, priority date: 11.06.97, filing date: 10.06.98; WO-A-99/11790, publication date: 11.03.99, priority dates: 04.09.97 and 04.09.07, filing date: 03.09.98; WO-A-99/31128, publication date: 24.06.99, priority date: 16.12.97, filing date: 02.12.98.

5. Additional remarks to item VIII :

The following objections are, however, raised under **Article 6 PCT** concerning the clarity of the claims :

i) according to Article 6 PCT in combination with Rule 6.3 PCT the claims shall define the matter for which protection is sought in terms of technical features. The IPEA considers that a peptide, polypeptide, protein, oligonucleotide, gene, etc.. being chemical products must be clearly and unambiguously characterized by their amino acid and/or nucleic acid

sequences, i.e. by reference to their specific SEQ ID No. The characterization of a product only by the desired function or by an arbitrary abbreviation, such as DETH, without any real technical meaning does not fulfill the requirements of said Article 6 PCT in combination with Rule 6.3 PCT. Thus, claims 1-4, 6 and 8-9 (as well as claim 7 as far as directly dependent on claims 1-4) do not fulfil these requirements.

ii) in this respect, the "fragment" of claim 6 is only functionally defined by the result desired to be achieved, namely the induction of apoptosis, but without any further technical parameter (contrary to the description, wherein it is said that part of the intracellular carboxyl terminus (residues 121-405 of SEQ ID No.: 2) contains the death region able to induce apoptosis (residues 168-240 of SEQ ID No.: 2), page 4, line 13 and page 5 line 11).

iii) the attention of the Applicant is also drawn to the fact that almost any protein contains portions or domains (as well as small, arbitrary peptides that can be also used as haptens), which can have a significant homology to similar domains of other completely unrelated proteins (apart from the presence of said domains). These domains do comprise immunoepitopes which can elicit antibodies that (more or less) cross-react with these unrelated proteins. The production of "specific or selective" antibodies requires the identification of "specific, selective or unique" immunoepitopes, the production of antibodies directed against said immunoepitope, the demonstration that said antibodies are able to successfully react against the whole protein (containing such immunoepitopes. It could well be that structural constraints hinder the identification of said immunoepitopes when present in the whole protein) and that they are actually "specific" (and relevant) of said protein. Therefore, the IPEA considers that as far as said "specific" epitopes are not explicitly disclosed in the description or else the production of said "specific" antibodies is not clearly exemplified in the description (example 5 only discloses the production of anti-DETH polyclonal antibodies but it is silent in respect of their specificity), such a subject matter is only worded in terms of the result to be achieved and it can not be seen as being **fully** (i.e. formally and technically) supported by the description as required by Article 6 PCT. Furthermore, in view of the significant homology between the DETH and other members of the known TNFR1/FAS/NGFR family receptors, and in particular in certain domains such as the death domain, the transmembrane domain, etc... the IPEA considers that the "specific" epitopes should be clearly and explicitly defined in those claims. Thus, the subject matter directed to a general "antibody specific" for the claimed DETH, namely claim 8 does not fulfil these requirements.

**INTERNATIONAL PRELIMINARY
EXAMINATION REPORT - SEPARATE SHEET**

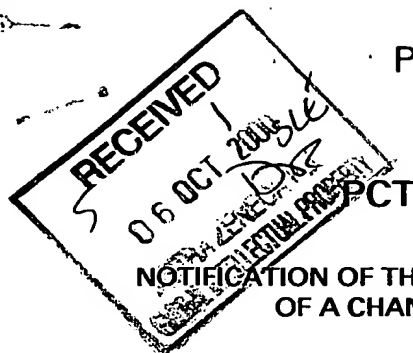
International application No. PCT/GB99/01793

iv) the subject matter of claim 9 refers to "a method of inducing apoptosis ... expressing DETH in a cell...". The IPEA considers that not any cell would be suitable for apoptosis and would have the appropriate cellular machinery for recognizing the function of the disclosed DETH protein. In fact, the application has shown that such a method would only be successfully carried out in "mammalian cells" (page 3, line 20; page 12, lines 16-19, etc...).

v) the IPEA considers that there are several inconsistencies between the embodiments of the invention as described in the description (methods identification antagonists, DETH antagonists, etc...) and the actual claimed subject matter in the set of claims (PCT Gazette, 29.10.98, PCT International Preliminary Examination Guidelines, Section IV, III-4.3).

PATENT COOPERATION TREATY 09 / 733956

COPY TO DEG



(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

From the INTERNATIONAL BUREAU

To:

GILES, Allen, Frank
AstraZeneca
Global Intellectual Property
P.O. Box 272
Mereside, Alderley Park
Macclesfield, Cheshire SK10 4GR
ROYAUME-UNI

Date of mailing (day/month/year) 28 September 2000 (28.09.00)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference PHM 70311/WO	
International application No. PCT/GB99/01793	International filing date (day/month/year) 08 June 1999 (08.06.99)

1. The following indications appeared on record concerning:

☐ the applicant ☐ the inventor ☒ the agent ☐ the common representative

Name and Address GILES, Allen, Frank AstraZeneca UK Limited Global Intellectual Property Mereside, Alderley Park Macclesfield Cheshire SK10 4TG United Kingdom	State of Nationality	State of Residence
	Telephone No. (01625) 516573	
	Facsimile No. (01625) 583358	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address GILES, Allen, Frank AstraZeneca Global Intellectual Property P.O. Box 272 Mereside, Alderley Park Macclesfield, Cheshire SK10 4GR United Kingdom	State of Nationality	State of Residence
	Telephone No. 01625 516573	
	Facsimile No. 01625 583358	
	Teleprinter No.	

3. Further observations, if necessary:

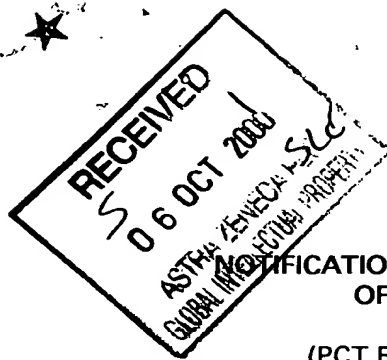
4. A copy of this notification has been sent to:

☒ the receiving Office ☐ the designated Offices concerned
☐ the International Searching Authority ☒ the elected Offices concerned
☒ the International Preliminary Examining Authority ☐ other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland Facsimile No.: (41-22) 740.14.35	Authorized officer Athina Nickitas-Etienne Telephone No.: (41-22) 338.83.38
---	---

PATENT COOPERATION TREATY **09 / 733956**

COPY TO PEG



PCT

From the INTERNATIONAL BUREAU

To:

GILES, Allen, Frank
AstraZeneca
Global Intellectual Property
P.O. Box 272
Mereside, Alderley Park
Macclesfield, Cheshire SK10 4GR
ROYAUME-UNI

NOTIFICATION OF THE RECORDING
OF A CHANGE

(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

Date of mailing (day/month/year) 28 September 2000 (28.09.00)	IMPORTANT NOTIFICATION
Applicant's or agent's file reference PHM 70311/WO	
International application No. PCT/GB99/01793	International filing date (day/month/year) 08 June 1999 (08.06.99)

1. The following indications appeared on record concerning:

☒ the applicant ☐ the inventor ☐ the agent ☐ the common representative

Name and Address ASTRAZENECA UK LIMITED 15 Stanhope Gate London W1Y 6LN United Kingdom	State of Nationality GB	State of Residence GB
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☒ the person ☒ the name ☒ the address ☒ the nationality ☒ the residence

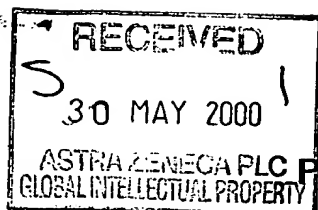
Name and Address ASTRAZENECA AB <i>OR</i> S-151 85 Södertälje Sweden	State of Nationality SE	State of Residence SE
	Telephone No.	
	Facsimile No.	
	Teleprinter No.	

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office ☐ the designated Offices concerned
☐ the International Searching Authority ☒ the elected Offices concerned
☒ the International Preliminary Examining Authority ☐ other:

The International Bureau of WIPO 34, chemin des Colombettes 1211 Geneva 20, Switzerland	Authorized officer Athina Nickitas-Etienne
Facsimile No.: (41-22) 740.14.35	Telephone No.: (41-22) 338.83.38



PATENT COOPERATION TREATY 09 / 733956

From the INTERNATIONAL BUREAU

To:

GILES, Allen, Frank
 AstaZeneca UK Limited
 Global Intellectual Property
 Mereside, Alderley Park
 Macclesfield
 Cheshire SK10 4TG
 ROYAUME-UNI

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and
 Administrative Instructions, Section 422)

Date of mailing (day/month/year) 22 May 2000 (22.05.00)	
Applicant's or agent's file reference PHM 70311/WO	IMPORTANT NOTIFICATION
International application No. PCT/GB99/01793	International filing date (day/month/year) 08 June 1999 (08.06.99)

1. The following indications appeared on record concerning:

☒ the applicant ☐ the inventor ☐ the agent ☐ the common representative

Name and Address

ZENECA LIMITED
 15 Stanhope Gate
 London W1Y 6LN
 United Kingdom

State of Nationality

GB

State of Residence

GB

Telephone No.

Facsimile No.

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☒ the person ☐ the name ☐ the address ☐ the nationality ☐ the residence

Name and Address

ASTRAZENECA UK LIMITED
 15 Stanhope Gate
 London W1Y 6LN
 United Kingdom

State of Nationality

GB

State of Residence

GB

Telephone No.

Facsimile No.

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office ☐ the designated Offices concerned
☐ the International Searching Authority ☒ the elected Offices concerned
☒ the International Preliminary Examining Authority ☐ other:

The International Bureau of WIPO
 34, chemin des Colombettes
 1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

H. Zhou

Telephone No.: (41-22) 338.83.38

RECEIVED

30 MAY 2000

ASTAZENeca PLC
GLOBAL INTELLECTUAL PROPERTY

PATENT COOPERATION TREATY 09/722956

PCT

From the INTERNATIONAL BUREAU

To:

GILES, Allen, Frank
AstaZeneca UK Limited
Global Intellectual Property
Mereside, Alderley Park
Macclesfield
Cheshire SK10 4TG
ROYAUME-UNI

NOTIFICATION OF THE RECORDING OF A CHANGE

(PCT Rule 92bis.1 and
Administrative Instructions, Section 422)

Date of mailing (day/month/year) 22 May 2000 (22.05.00)	
Applicant's or agent's file reference PHM 70311/WO	IMPORTANT NOTIFICATION
International application No. PCT/GB99/01793	International filing date (day/month/year) 08 June 1999 (08.06.99)

1. The following indications appeared on record concerning:

☐ the applicant ☐ the inventor ☒ the agent ☐ the common representative

Name and Address

GILES, Allen, Frank
AstaZeneca PLC
Global Intellectual Property
Mereside, Alderley Park
Macclesfield
Cheshire SK10 4TG
United Kingdom

State of Nationality

State of Residence

Telephone No.

(01625) 516573

Facsimile No.

(01625) 583358

Teleprinter No.

2. The International Bureau hereby notifies the applicant that the following change has been recorded concerning:

☐ the person ☐ the name ☒ the address ☐ the nationality ☐ the residence

Name and Address

GILES, Allen, Frank
AstaZeneca UK Limited
Global Intellectual Property
Mereside, Alderley Park
Macclesfield
Cheshire SK10 4TG
United Kingdom

State of Nationality

State of Residence

Telephone No.

(01625) 516573

Facsimile No.

(01625) 583358

Teleprinter No.

3. Further observations, if necessary:

4. A copy of this notification has been sent to:

☒ the receiving Office ☐ the designated Offices concerned
☐ the International Searching Authority ☒ the elected Offices concerned
☒ the International Preliminary Examining Authority ☐ other:

The International Bureau of WIPO
34, chemin des Colombettes
1211 Geneva 20, Switzerland

Facsimile No.: (41-22) 740.14.35

Authorized officer

H. Zhou

Telephone No.: (41-22) 338.83.38

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference PHM 70311/WO	FOR FURTHER ACTION see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.	
International application No. PCT/GB 99/ 01793	International filing date (day/month/year) 08/06/1999	(Earliest) Priority Date (day/month/year) 12/06/1998
Applicant ZENECA LIMITED et al.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 4 sheets.

☒ It is also accompanied by a copy of each prior art document cited in this report.

1. Basis of the report

a. With regard to the **language**, the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

☒ contained in the international application in written form.

☒ filed together with the international application in computer readable form.

☐ furnished subsequently to this Authority in written form.

☐ furnished subsequently to this Authority in computer readable form.

☐ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

☐ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☐ **Unity of invention is lacking** (see Box II).

4. With regard to the **title**,

☐ the text is approved as submitted by the applicant.

☒ the text has been established by this Authority to read as follows:

TNFR-LIKE PROTEIN WITH DEATH DOMAIN

5. With regard to the **abstract**,

☒ the text is approved as submitted by the applicant.

☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

☐ as suggested by the applicant.

☐ because the applicant failed to suggest a figure.

☐ because this figure better characterizes the invention.

☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 99/ 01793

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 9 can be understood to comprise methods in vivo and is therefore considered a method of treatment of the human/animal body, the search has been limited to the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/01793

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/705 C07K16/28 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	PAN G. ET AL.: "Identification and functional characterization of DR6, a novel death domain-containing TNF receptor" FEBS LETTERS, vol. 431, 1998, pages 351-356, XP002113408 the whole document ---	1-9
P,X	WO 99 15663 A (MILLENNIUM BIOTHERAPEUTICS INC) 1 April 1999 (1999-04-01) sequence of TRL II ---	1-9
P,X	EP 0 869 179 A (SMITHKLINE BEECHAM CORP) 7 October 1998 (1998-10-07) sequence of TR7 --- -/--	1-9

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

26 August 1999

Date of mailing of the international search report

06/09/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2
NL - 2280 HV Rijswijk
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,
Fax: (+31-70) 340-3016

Authorized officer

Galli, I

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/01793

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P, X	WO 98 56892 A (HUMAN GENOME SCIENCES INC ;NI JIAN (US); FAN PING (US); GENTZ REIN) 17 December 1998 (1998-12-17) sequence of TR9 ----	1-9
P, X	WO 99 11790 A (ZYMOGENETICS INC) 11 March 1999 (1999-03-11) sequence of ZTNFR-6 ----	1-9
E	WO 99 31128 A (INCYTE PHARMA INC ;AU YOUNG JANICE (US); BANDMAN OLGA (US); KASER) 24 June 1999 (1999-06-24) seq. ID 4 ----	1-9
A	KITSON J ET AL: "A DEATH-DOMAIN-CONTAINING RECEPTOR THAT MEDIATES APOPTOSIS" NATURE, vol. 384, no. 6607, 28 November 1996 (1996-11-28), pages 372-375, XP002036560 ISSN: 0028-0836 abstract ----	1-9
A	CHINNAIYAN A M ET AL: "SIGNAL TRANSDUCTION BY DR3, A DEATH DOMAIN-CONTAINING RECEPTOR RELATED TO TNFR-1 AND CD95" SCIENCE, vol. 274, no. 5289, 8 November 1996 (1996-11-08), pages 990-992, XP000676685 ISSN: 0036-8075 abstract ----	1-9
A	PAN G ET AL: "AN ANTAGONIST DECOY RECEPTOR AND A DEATH DOMAIN-CONTAINING RECEPTOR FOR TRAIL" SCIENCE, vol. 277, 8 August 1997 (1997-08-08), pages 815-818, XP002065147 ISSN: 0036-8075 abstract ----	1-9
A	PAN G ET AL: "THE RECEPTOR FOR THE CYTOTOXIC LIGAND TRAIL" SCIENCE, vol. 276, 4 April 1997 (1997-04-04), pages 111-113, XP002913702 ISSN: 0036-8075 abstract -----	1-9

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/01793

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9915663	A	01-04-1999	AU	9777698 A	12-04-1999
EP 0869179	A	07-10-1998	JP	11018786 A	26-01-1999
WO 9856892	A	17-12-1998	AU	7830398 A	30-12-1998
WO 9911790	A	11-03-1999	AU	9130598 A	22-03-1999
WO 9931128	A	24-06-1999	NONE		

PCT

REQUEST

The undersigned requests that the present international application be processed according to the Patent Cooperation Treaty.

For receiving Office use only

International Application No.

International Filing Date

Name of receiving Office and "PCT International Application"

Applicant's or agent's file reference
(if desired) (12 characters maximum) PHM 70311/WO

Box No. I TITLE OF INVENTION

PROTEIN

Box No. II APPLICANT

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

ZENECA Limited
15 Stanhope Gate
LONDON
W1Y 6LN
GB

☐ This person is also inventor.

Telephone No.

(01625) 516173

Facsimile No.

(01625) 583358

Teleprinter No.

669095/669388

State (that is, country) of nationality:

GB

State (that is, country) of residence:

GB

This person is applicant
for the purposes of:

☐ all designated
States

☒ all designated States except
the United States of America

☐ the United States
of America only

☐ the States indicated in
the Supplemental Box

Box No. III FURTHER APPLICANT(S) AND/OR (FURTHER) INVENTOR(S)

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

LU, Jian Jason
1800 Concord Pike
P O Box 15437
Wilmington
Delaware
USA

This person is:

☐ applicant only

☒ applicant and inventor

☐ inventor only (If this check-box
is marked, do not fill in below.)

State (that is, country) of nationality:

CN

State (that is, country) of residence:

US

This person is applicant
for the purposes of:

☐ all designated
States

☐ all designated States except
the United States of America

☒ the United States
of America only

☐ the States indicated in
the Supplemental Box

☒ Further applicants and/or (further) inventors are indicated on a continuation sheet.

Box No. IV AGENT OR COMMON REPRESENTATIVE; OR ADDRESS FOR CORRESPONDENCE

The person identified below is hereby/has been appointed to act on behalf
of the applicant(s) before the competent International Authorities as:

☒ agent

☐ common representative

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country.)

GILES, Allen Frank
Intellectual Property Department
ZENECA Pharmaceuticals
Mereside, Alderley Park,
Macclesfield, Cheshire, SK10 4TG, GB

Telephone No.

(01625) 516573

Facsimile No.

(01625) 583358

Teleprinter No.

669095/669388

☐ Address for correspondence: Mark this check-box where no agent or common representative is/has been appointed and the space above is used instead to indicate a special address to which correspondence should be sent.

Continuation of Box No. III FURTHER APPLICANTS AND/OR (FURTHER) INVENTORS

If none of the following sub-boxes is used, this sheet should not be included in the request.

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

GOMES, Bruce Charles
1800 Concord Pike
P O Box 15437
Wilmington
Delaware
USA

This person is:

- ☐ applicant only
☒ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:
US

State (that is, country) of residence:
US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

FIELES, William Edward
1800 Concord Pike
P O Box 15437
Wilmington
Delaware
USA

This person is:

- ☐ applicant only
☒ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:
US

State (that is, country) of residence:
US

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☒ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

- ☐ applicant only
☐ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

Name and address: (Family name followed by given name; for a legal entity, full official designation. The address must include postal code and name of country. The country of the address indicated in this Box is the applicant's State (that is, country) of residence if no State of residence is indicated below.)

This person is:

- ☐ applicant only
☐ applicant and inventor
☐ inventor only (If this check-box is marked, do not fill in below.)

State (that is, country) of nationality:

State (that is, country) of residence:

This person is applicant for the purposes of: ☐ all designated States ☐ all designated States except the United States of America ☐ the United States of America only ☐ the States indicated in the Supplemental Box

☐ Further applicants and/or (further) inventors are indicated on another continuation sheet.

Box No.V DESIGNATION OF STATES

The following designations are hereby made under Rule 4.9(a) (mark the applicable check-boxes: at least one must be marked):

Regional Patent

- ☒ **AP ARIPO Patent:** GH Ghana, GM Gambia, KE Kenya, LS Lesotho, MW Malawi, SD Sudan, SZ Swaziland, UG Uganda, ZW Zimbabwe, and any other State which is a Contracting State of the Harare Protocol and of the PCT
- ☒ **EA Eurasian Patent:** AM Armenia, AZ Azerbaijan, BY Belarus, KG Kyrgyzstan, KZ Kazakhstan, MD Republic of Moldova, RU Russian Federation, TJ Tajikistan, TM Turkmenistan, and any other State which is a Contracting State of the Eurasian Patent Convention and of the PCT
- ☒ **EP European Patent:** AT Austria, BE Belgium, CH and LI Switzerland and Liechtenstein, CY Cyprus, DE Germany, DK Denmark, ES Spain, FI Finland, FR France, GB United Kingdom, GR Greece, IE Ireland, IT Italy, LU Luxembourg, MC Monaco, NL Netherlands, PT Portugal, SE Sweden, and any other State which is a Contracting State of the European Patent Convention and of the PCT
- ☒ **OA OAPI Patent:** BF Burkina Faso, BJ Benin, CF Central African Republic, CG Congo, CI Côte d'Ivoire, CM Cameroon, GA Gabon, GN Guinea, ML Mali, MR Mauritania, NE Niger, SN Senegal, TD Chad, TG Togo, and any other State which is a member State of OAPI and a Contracting State of the PCT (if other kind of protection or treatment desired, specify on dotted line)

National Patent (if other kind of protection or treatment desired, specify on dotted line):

- | | |
|--|--|
| <input checked="" type="checkbox"/> AL Albania | <input checked="" type="checkbox"/> LS Lesotho |
| <input checked="" type="checkbox"/> AM Armenia | <input checked="" type="checkbox"/> LT Lithuania |
| <input checked="" type="checkbox"/> AT Austria | <input checked="" type="checkbox"/> LU Luxembourg |
| <input checked="" type="checkbox"/> AU Australia | <input checked="" type="checkbox"/> LV Latvia |
| <input checked="" type="checkbox"/> AZ Azerbaijan | <input checked="" type="checkbox"/> MD Republic of Moldova |
| <input checked="" type="checkbox"/> BA Bosnia and Herzegovina | <input checked="" type="checkbox"/> MG Madagascar |
| <input checked="" type="checkbox"/> BB Barbados | <input checked="" type="checkbox"/> MK The former Yugoslav Republic of Macedonia |
| <input checked="" type="checkbox"/> BG Bulgaria | <input checked="" type="checkbox"/> MN Mongolia |
| <input checked="" type="checkbox"/> BR Brazil | <input checked="" type="checkbox"/> MW Malawi |
| <input checked="" type="checkbox"/> BY Belarus | <input checked="" type="checkbox"/> MX Mexico |
| <input checked="" type="checkbox"/> CA Canada | <input checked="" type="checkbox"/> NO Norway |
| <input checked="" type="checkbox"/> CH and LI Switzerland and Liechtenstein | <input checked="" type="checkbox"/> NZ New Zealand |
| <input checked="" type="checkbox"/> CN China | <input checked="" type="checkbox"/> PL Poland |
| <input checked="" type="checkbox"/> CU Cuba | <input checked="" type="checkbox"/> PT Portugal |
| <input checked="" type="checkbox"/> CZ Czech Republic | <input checked="" type="checkbox"/> RO Romania |
| <input checked="" type="checkbox"/> DE Germany | <input checked="" type="checkbox"/> RU Russian Federation |
| <input checked="" type="checkbox"/> DK Denmark | <input checked="" type="checkbox"/> SD Sudan |
| <input checked="" type="checkbox"/> EE Estonia | <input checked="" type="checkbox"/> SE Sweden |
| <input checked="" type="checkbox"/> ES Spain | <input checked="" type="checkbox"/> SG Singapore |
| <input checked="" type="checkbox"/> FI Finland | <input checked="" type="checkbox"/> SI Slovenia |
| <input checked="" type="checkbox"/> GB United Kingdom | <input checked="" type="checkbox"/> SK Slovakia |
| <input checked="" type="checkbox"/> GE Georgia | <input checked="" type="checkbox"/> SL Sierra Leone |
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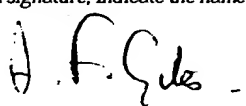
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(54) Title: TNFR-LIKE PROTEIN WITH DEATH DOMAIN		
(57) Abstract <p>The present invention provides a novel 45 kilodalton protein, <u>Death Domain Expressing Tumor Necrosis Factor Receptor Family Homologue</u>, referred to as DETH. DETH has a role in apoptosis and appears to be a member of the TNFR1/FAS/NGFR family of receptors. The present invention also provides nucleic acid sequences encoding DETH, fragments thereof or variants thereof and methods of using DETH or fragment thereof to identify compounds that inhibit or induce apoptosis.</p>		

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TNFR-LIKE PROTEIN WITH DEATH DOMAIN

Field of the Invention

The present invention relates to the field of apoptosis (programmed cell death). More particularly, the present invention relates to a novel 45 kilodalton protein, Death Domain Expressing Tumor Necrosis Factor Receptor Family Homologue, referred to as DETH, nucleic acid sequences encoding the protein and uses thereof.

Background of the Invention

Apoptotic neuronal death appears to be an important component of a number of common neurological and psychiatric disorders (Altman, J. (1992) Programmed cell death: the paths to suicide, TINS 15:278-280; Margolis, RL et al. (1994) Programmed cell death: implications for neuropsychiatric disorders, Biol Psychiatry 35:946-956; Cotman, CW and Anderson, AJ (1995) A potential role for apoptosis in neurodegeneration and Alzheimer's disease, Mol Neurobiol 10:19-45; and W.G. Tatton et al (1997) [suppl], Apoptosis in neurodegenerative disorders: potential for therapy by modifying gene transcription, J. Neural Transm 49:245-268). Some of those disorders include stroke, Alzheimer's disease, Parkinson's disease, Amyotrophic Lateral Sclerosis, hereditary retinal degenerations, glaucoma and spinal muscular. Apoptosis of glial cells may also contribute to disorders like multiple sclerosis and diabetic peripheral neuropathy. The role of apoptosis in neurodegenerative disorders has been reviewed by in W. G. Tatton et al. (1997) J. Neural Transm. [Suppl] 49:245-268.

The phenomenon of programmed cell death, or apoptosis is known to be involved in the normal course of a wide variety of developmental processes including immune and nervous system maturation. Apoptosis also plays a role in adult tissues having high cell turnover rates. A number of different physiological signals normally activate apoptosis in these contexts, but nonphysiological insults such as irradiation and exposure to drugs such as many in current use in chemotherapy which damage DNA also can trigger apoptosis. Glucocorticoids, withdrawal of cytokines, DNA damage and signals through the antigen receptors of T or B lymphocytes can all induce apoptosis, depending on the inherent susceptibility of each individual cell type. In addition, signals through death receptors located

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in the plasma membrane of many cells can potently induce apoptosis. Two surface death receptors have been identified, tumor necrosis factor receptor 1 (TNF-R1) and Fas receptor (FasR).

Apoptosis has been implicated as an important cellular safeguard against tumorigenesis. Under certain conditions, cells die by apoptosis in response to high-level or deregulated expression of oncogenes. Suppression of the apoptotic program by a variety of genetic lesions may contribute to the development and progression of malignancies. This is well-illustrated by the frequent mutation of the p53 tumor suppresser gene in human tumors. Wild-type p53 is required for efficient induction of apoptosis following DNA damage and cell death induced by constitutive expression of certain oncogenes. The cytotoxicity of many commonly used chemotherapeutic agents is mediated by wild-type p53. This loss of p53 function may contribute to the clinically significant problem of drug resistant tumor cells emerging following chemotherapy regimens.

At the heart of the apoptosis program lies a family of cysteine proteases, which cleave specifically at aspartic acid (aspase activity). "Caspase" is derived from cysteine protease with aspase activity. The caspases are divided into distinct subfamilies, those related to interleukin-1 beta-converting enzyme (ICE) and those related to CED-3, a protein required for cell suicide in *C. elegans*. At least some of the caspases appear to act upon each other in a proteolytic cascade. Caspases are proenzymes achieving enzymatic potential only after reassembly of their active subunits.

Once the cascade of caspases have been activated, a series of degradative proteolytic events occur in the cell. Caspase-3 (CPP32) is suspected to cleave the retinoblastoma protein, thereby disrupting the cell cycle. Both caspase-3 and caspase-1 (ICE) cleave poly-ADP ribose polymerase (PARP), disrupting DNA repair, while caspase-6 (Mch-2) cleaves laminins. Interestingly, the T lymphocyte-produced death-inducing molecule granzyme B is also an aspase, and is known to cleave caspases 3, 6, 7, 8, 9 and 10 in a perforin-dependent manner. Similarly, granzyme A has been reported to have ICE-like activity.

Fas receptor (FasR), tumor necrosis factor receptor-1 (TNF-R1), FADD and TRADD each contain a death domain which is capable of self-association. Death domain:death domain self-interactions are involved in transmitting signals from plasma membrane receptors to down-stream signaling events. Ligation of FasR by Fas ligand (FasL) or TNF-R1 by TNF,

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presumably alters the conformation of FasR or TNF-R1 death domains, respectively.

Through the self-interactions of death domains FADD is able to recognize conformationally-activated FasR, while TRADD recognizes activated TNF-R1. Activation of FADD induces collaboration of its death effector domain with FLICE (caspase-8) and/or FLICE2, FLICE, in turn, is thought to cleave and activate caspase-1, while FLICE2 activates caspase-2 and cleaves PARP. Each of these activating steps directs the cell towards its own self-destruction. Activation of TRADD triggers a cascade resulting in activation and nuclear translocation of transcription factors, such as NK-KB, as well as the JUN oncogenes. The death domains of FADD and TRADD have been found to interact, indicating that cross-talk may occur between the FADD and TRADD signaling pathways.

Apoptosis is an important component of a number of common neurological and psychiatric disorders and cancers. Understanding the phenomenon of programmed cell death and providing treatments for such disorders and diseases and methods for identifying therapeutics to treat such disorders and diseases is clearly desirable.

Summary of the Invention

The present invention provides a novel protein, Death Domain Expressing Tumor Necrosis Factor Receptor Family Homologue, referred to as DETH. DETH has a role in apoptosis and appears to be a member of the TNFR1/FAS/NGFR family of receptors.

Overexpression of DETH in mammalian cells induces apoptosis. DETH or variant thereof or fragment thereof may be useful in the treatment of cancer or autoimmune diseases where induction of apoptosis is desirable. DETH or variant thereof or fragment thereof is also useful for screening to identify inhibitors of apoptosis or agonists of apoptosis. Inhibitors of apoptosis would be useful, for example, in the treatment of neurodegenerative diseases where inhibition of apoptosis is desirable. Agonists of apoptosis would be useful in the treatment of cancer or autoimmune disease where induction of apoptosis is desirable.

The present invention also provides nucleic acid sequences encoding DETH, fragments thereof or variants thereof.

Another aspect of the invention provides methods of using DETH or fragment thereof to identify compounds that inhibit or induce apoptosis.

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A further aspect of the invention provides methods, reagents and kits for detecting DETH or nucleic acid encoding DETH in cells, body fluids or tissues.

Yet another aspect of the invention provides pharmaceutical compositions comprising DETH or variant thereof or fragment thereof, nucleic acid encoding DETH or variant thereof
5 or fragment thereof, or antibody specific for DETH and a pharmaceutically acceptable carrier or diluent.

These and other aspects of the present invention are described in the following detailed description.

10 **Detailed Description of the Invention**

The present invention provides a novel purified 45 kilodalton protein, Death Domain
Expressing Tumor Necrosis Factor Receptor Family Homologue, referred to as DETH (SEQ
ID NO:2). DETH contains a death domain or region in the intracellular carboxyl terminus
followed by a fifteen amino acid transmembrane region and an amino terminal extracellular.
15 DETH appears to be a member of the TNFR1/FAS/NGFR family of receptors. Without
wishing to be bound by any particular mode or theory of action, it is presently believed that
DETH acts in the early stages of apoptosis. Transient expression of DETH in human cell
lines HeLa, NT2 and A549 caused cells to die of apoptosis, i.e. morphologically cells which
expressed DETH protein were rounded up, and DNA in the nucleus was condensed and
20 disintegrated.

In addition to the purified full length protein, the present invention also encompasses
DETH variants. A preferred DETH variant is a purified protein having at least 80% amino
acid sequence identity to the DETH amino acid sequence (SEQ ID NO: 2). More preferably
such DETH variant is one having at least 90% amino acid sequence identity, most preferably
25 such DETH variant is one having at least 95% amino acid sequence identity to the DETH
amino acid sequence. Especially preferred variants are those having at least 99% amino acid
sequence identity to DETH.

A variant of DETH may have an amino acid sequence that is different by one or more
amino acid substitutions. The variant may have conservative changes, wherein a substituted
30 amine acid has similar structural or chemical properties, e.g., replacement of leucine with
isoleucine. More rarely, a variant may have nonconservative changes, e.g., replacement of a

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glycine with tryptophan. Similar minor variations may also include amino acid deletions of from one to about five amino acids or insertions of from one to about five amino acids, or both. Guidance in determining which and how many amino acid residues may be substituted, inserted or deleted without abolishing biological or immunological activity may be found using computer programs well known in the art, for example, DNASTar software.

The present invention also provides fragments of DETH. Such fragments can be of any length which is shorter than the full length protein. Fragments include portions of the full length protein capable of inducing apoptosis. Such fragments can be obtained by testing of fragments of DETH in assays such as those disclosed herein to identify those fragments capable of inducing apoptosis. Fragments can also include the various regions of DETH, such as the transmembrane region (amino acids 103-118), the death region (amino acids 168-240), the serine-proline rich region (amino acids 262-325) the extracellular region (amino acids 1-102) and the intracellular region (amino acids 121-405). Other fragments include portions of the protein capable of binding to cellular ligands, and portions of the protein capable of eliciting an immune response in a mammal. Fragments of DETH can be in a purified condition and may also show deletions, insertions or substitutions of amino acids as described herein for the full length protein.

The DETH protein may also show deletions, insertions or substitutions of amino acid residues which produce a silent change and result in a functionally equivalent DETH protein. Deliberate amino acid substitutions may be made on the basis of similarity in polarity, charge, solubility, hydrophobicity, hydrophilicity, and/or the amphipathic nature of the residues as long as the biological activity of DETH is retained. For example, negatively charged amino acids include aspartic acid and glutamic acid; positively charged amino acids include lysine and arginine; and amino acids with uncharged polar head groups having similar hydrophilicity values include leucine, isoleucine, valine, glycine, alanine; asparagine, serine, threonine, phenylalanine, and tyrosine.

A deletion refers to a change in either nucleotide or amino acid sequence in which one or more nucleotides or amino acid residues, respectively, are absent.

An insertion or addition refers to change in a nucleotide or amino acid sequence which has resulted in the addition of one or more nucleotides or amino acid residues, respectively, as compared to the naturally occurring DETH.

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A substitution results from the replacement of one or more nucleotides or amino acids by different nucleotides or amino acids, respectively.

The present invention also provides purified nucleic acid sequences encoding DETH, variant thereof or fragment thereof. A preferred sequence encoding DETH is shown in SEQ ID NO:1. Nucleic acid sequence as used herein refers to an oligonucleotide, nucleotide or polynucleotide and fragments or portions thereof and to DNA or RNA of genomic or synthetic origin which may be single- or double-stranded, and represent the sense or antisense strand. A nucleic acid sequence encoding DETH can be obtained from mammalian cells, preferably human cells using polymerase chain reaction techniques (PCR) and appropriate primers encompassing the 3' and 5' ends of the sequence encoding DETH shown in SEQ ID NO:1 with the nucleic acid in the cells serving as a template. A nucleic acid sequence encoding DETH was obtained from clone g1727750 obtained from the Merck/Washington University EST Project, St. Louis, Missouri, USA. Clone g1727750 was made from mRNA of a human colon epithelial cell line. Suitable primers for PCR include 5' primer - ttt aga tct atg aac tca aca gaa tcc aac tct tct gcc and 3' primer - gtc gac cta cag cag gtc agg aag atg gct ata aac ag . Suitable polymerase reaction conditions are those generally found in the art and exemplified herein in the examples.

The term biologically active refers to a DETH protein having structural, regulatory or biochemical functions of the naturally occurring DETH protein. Likewise immunologically active defines the capability of the natural, recombinant or synthetic DETH, or any fragment thereof, to induce a specific immune response in appropriate animals or cells and to bind with special antibodies.

The term derivative as used herein refers to the chemical modification of nucleic acid encoding DETH variant or fragment, or the encoded DETH protein, variant or fragment. Illustrative of such modifications would be replacement of hydrogen by an alkyl, aryl, or amino group. A DETH derivative would encode a protein which retains biological characteristics and/or activity of natural DETH.

As used herein, the term purified refers to molecules, either nucleic acid or amino acid sequences, that are removed from their natural environment and isolated or separated from at least one other component with which they are naturally associated.

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A nucleic acid sequence, or portions thereof, encoding DETH and its derivatives may be produced entirely by synthetic chemistry, after which the synthetic gene may be inserted into any of the many available DNA vectors and cell systems using reagents that are well known in the art. Moreover, synthetic chemistry may be used to introduce mutations into a sequence encoding DETH or any fragment thereof.

Also included within the scope of the present invention are nucleotide sequences that are capable of hybridizing to the nucleotide sequence of DETH (SEQ ID NO:1) under various conditions of stringency. Hybridization conditions are based on the melting temperature (T_m) of the nucleic acid binding complex or probe, as taught, for example, in Berger and Kimmer (1987, *Guide to Molecular Cloning Techniques, Methods in Enzymology*, Vol 152, Academic Press, San Diego Calif.) and may be used at a defined "stringency" as explained below.

Maximum stringency typically occurs at about $T_m - 5^\circ\text{C}$. (5°C below the T_m of the probe); "high stringency" at about 5°C . to 10°C . below T_m ; "intermediate stringency" at about 10°C to 20°C . below T_m ; and "low stringency" at about 20°C to 25°C . below T_m .

As will be understood by those of skill in that art, a maximum stringency hybridization can be used to identify or detect identical polynucleotide sequences, while an intermediate (or low) stringency hybridization can be used to identify or detect similar or related polynucleotide sequences.

The term hybridization as used herein includes any process by which a strand of nucleic acid joins with a complementary strand through base pairing (Coombs J (1994) *Dictionary of Biotechnology*, Stockton Press, New York, N.Y.). Amplification as carried out in the polymerase chain reaction technologies may follow the process of hybridization.

Included within the scope of the present invention are alleles of DETH. As used herein, an allele or allelic sequence is an alternative form of DETH. Alleles result from a mutation, i.e., a change in the nucleic acid sequence, and generally produce altered mRNAs or polypeptides whose structure or function may or may not be altered. Any given gene may have none, one or many allelic forms. Common mutational changes which give rise to alleles are generally ascribed to natural deletions, additions or substitutions of amino acids. Each of these types of changes may occur alone, or in combination with the others, one or more times in a given sequence.

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Methods for DNA sequencing are well known in the art and employ such enzymes as the Klenow fragment of DNA polymerase I, Sequenase® (US Biochemical Corp, Cleveland Ohio)), Taq polymerase (Perkin Elmer, Norwalk Conn.), thermostable T7 polymerase (Amersham, Chicago, Ill.), or combinations of recombinant polymerases and proofreading exonucleases such as the ELONGASE Amplification System marked by Gibco BRL (Gaithersburg Md.). Preferably, the process is automated with machines such as the Hamilton Micro Lab 2200 (Hamilton, Reno Nev.), Peltier Thermal Cycler (PTC200; M J Research, Watertown Mass.) and the ABI377 DNA sequencers (Perkin Elmer).

In accordance with the present invention, nucleic acid sequences which encode DETH, fragments, fusion proteins or functional equivalents thereof may be used in recombinant DNA molecules that direct the expression of DETH in appropriate host cells. Due to the inherent degeneracy of the genetic code, other DNA sequences which encode substantially the same or a functionality equivalent amino acid sequence, may be used to clone and express DETH. As will be understood by those of skill in the art, it may be advantageous to produce DETH encoding nucleotide sequences that possess non-naturally occurring codons. Codons preferred by a particular prokaryotic or eukaryotic host can be selected, for example, to increase the rate of DETH expression or to produce recombinant RNA transcripts having desirable properties, such as a longer half-life, than transcripts produced from naturally occurring sequence.

The nucleotide sequences of the present invention can be engineered in order to alter DETH coding sequence for a variety of reasons, including but not limited to, alterations which modify the cloning, processing and/or expression of the gene product. For example, mutations may be introduced using techniques which are well known in the art, such as site-directed mutagenesis to insert new restriction sites, to alter glycosylation patterns, to change codon preference, to produce splice variants, etc.

In another embodiment of the invention, a nucleic acid sequence encoding DETH or fragment thereof DETH may be ligated to a heterologous sequence to encode a fusion protein. For example, for screening of peptide libraries for inhibitors of DETH activity, it may be useful to encode a chimeric DETH protein that is recognized by a commercially available antibody. A fusion protein may also be engineered to contain a cleavage site located between a DETH sequence and the heterologous protein sequence, so that the DETH may be cleaved

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and purified away from the heterologous moiety. For example, a nucleic acid sequence encoding DETH or fragment thereof or variant thereof can be ligated to a sequence encoding glutathione-S-transferase (GST) to produce a GST/DETH fusion protein.

The coding sequence of DETH could be synthesized, whole or in part, using chemical methods well known in the art (see Caruthers M H et al (1980) Nuc Acids Res Symp Ser 215-23, Horn T et al (1980) Nuc Acids Res Symp Ser 225-32, etc.). Alternatively, the protein itself could be produced using chemical methods to synthesize a DETH amino acid sequence, whole or in part. For example, peptide synthesis can be performed using various solid-phase techniques well known in the art.

DETH or fragment thereof or variant thereof can be purified by methods known in the art for purification of peptides and proteins such as preparative high performance liquid chromatography and ion exchange chromatography. The composition of the synthetic peptides may be confirmed by amino acid analysis or sequencing. Additionally, the amino acid sequence of DETH, or any part thereof, may be altered during direct synthesis and/or combined using chemical methods with sequences from other proteins, or any part thereof, to produce a variant polypeptide.

A further aspect of the present invention provides vectors comprising a nucleic acid sequence encoding DETH, variant thereof or fragment thereof. In order to express a biologically active DETH, the nucleotide sequence encoding DETH or its functional equivalent, is inserted into an appropriate expression vector. Methods which are well known to those skilled in the art can be used to construct expression vectors containing a nucleic acid sequence encoding DETH, fragment or variant and appropriate transcriptional or translational controls. These methods include *in vitro* recombinant DNA techniques, synthetic techniques and *in vivo* recombination or genetic recombination. Such techniques are described in, for example, Sambrook et al (1989) *Molecular Cloning, A Laboratory Manual*, Cold Spring Harbor Press, Plainview N.Y. and Ausubel F M et al (1989) *Current Protocols in Molecular Biology*, John Wiley & Sons, New York, N.Y.

A variety of expression vector/host systems may be utilized to contain and express a DETH coding sequence. These include but are not limited to microorganisms such as bacteria transformed with recombinant bacteriophage, plasmid or cosmid DNA expression vectors; yeast transformed with yeast expression vectors; insect cell systems infected with virus

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expression vectors (e.g. baculovirus); plant cell systems transfected with virus expression vectors (e.g., cauliflower mosaic virus, CaMV; tobacco mosaic virus, TMV) or transformed with bacterial expression vectors (e.g. Ti or pBR322 plasmid); or animal cell systems.

The control elements or regulatory sequences of these systems vary in their strength and specificities and are those nontranslated regions of the vector, enhancers, promoters, and 3' untranslated regions, which interact with host cellular proteins to carry out transcription and translation. Depending on the vector system and host utilized, any number of suitable transcription and translation elements, including constitutive and inducible promoters, may be used. For example, when cloning in bacterial systems, inducible promoters such as the hybrid LacZ promoter of the Bluescript® phagemid (Stratagene, LaJolla Calif.) or pSport1 (Gibco BRL) and ptrp-lac hybrids may be used. The baculovirus polyhedrin promoter may be used in insect cells. Promoters or enhancers derived from the genomes of plant cells (e.g., heat shock, RUBISCO; and storage protein genes) or from plant viruses (e.g., viral promoters or leader sequences) may be cloned into the vector. In mammalian cell systems, promoters from mammalian genes or from mammalian viruses are most appropriate. If it is necessary to generate a cell line that contains multiple copies of DETH, vectors based on SV40 or EBV may be used with an appropriate selectable marker.

In bacterial systems, a number of expression vectors may be selected depending upon the use intended for DETH. For example, when large quantities of DETH are needed for the induction of antibodies, vectors which direct high level expression of fusion proteins that are readily purified may be desirable. Such vectors include, but are not limited to, the multifunctional *E. Coli* cloning and expression vectors such as Bluescript® (Stratagene), in which the DETH coding sequence may be ligated into the vector in frame with sequences for the amino-terminal Met and the subsequent 7 residues of β -galactosidase so that a hybrid protein is produced. pGEX vectors (Promega, Madison Wis.) may be used to express DETH or fragment or variant thereof as fusion proteins with glutathione S-transferase (GST). In general, such fusion proteins are soluble and can easily be purified from lysed cells by adsorption to glutathione-agarose beads followed by elution in the presence of free glutathione. Proteins made in such systems are designed to include heparin, thrombin or factor XA protease cleavage sites so that the cloned polypeptide of interest can be released from the GST moiety at will.

In the yeast, *Saccharomyces cerevisiae*, a number of vectors containing constitutive or inducible promoters such as alpha factor, alcohol oxidase and PGH may be used. For review, see Ausubel et al (supra) and Grant et al (1987) *Methods in Enzymology* 153:516-544).

In cases where plant expression vectors are used, the expression of a sequence
5 encoding DETH may be derived by any of a number of promoters. For example, viral promoters such as the 35S and 19S promoters of CaMV (Brisson et al (1984) *Nature* 310:511-514) may be used alone or in combination with the omega leader sequence from TMB (Takamatsu et al (1987) *EMBO J* 6:307-311). Alternatively, plant promoters such as the small subunit of RUBISCO (Coruzzi et al (1984) *EMBO J* 3:1671-1680; Broglie et al (1984)
10 *Science* 224:838-843); or heat shock promoters (Winter J and Sinibaldi iR M (1991) *Results Probl Cell Differ* 17:85-105) may be used. These constructs can be introduced into plant cells by direct DNA transformation or pathogen mediated transfection. For reviews of such techniques, see Hobbs S or Murry L E in McGraw Hill *Yearbook of Science and Technology* (1992) McGraw Hill New York, N.Y., pp 191-196 or Weissbach and Weissbach (1988)
15 *Methods for Plant Molecular Biology*, Academic Press, New York, N.Y., pp 421-463).

An alternative expression system which could be used to express DETH is an insect system. In one such system, *Autographa californica* nuclear polyhedrosis virus (AcNPV) is used as a vector to express foreign genes in *Spodoptera Frugiperda* cells or in *Trichoplusia* larvae. The DETH coding sequence may be cloned into a nonessential region of the virus,
20 such as, the polyhedrin gene, and placed under control of the polyhedrin promoter. Successful insertion of DETH will render the polyhedrin gene inactive and produce recombinant virus lacking coat protein. The recombinant viruses are then used to infect *S. frugiperda* cells or *Trichoplusia* larvae in which DETH is expressed (Smith et al (1983) *J Virol* 46:584; Engelhard E K et al (1994) *Proc Nat Acad Sci* 91:3224-7).

25 In mammalian host cells, a number of viral-based expression systems may be utilized. In cases where an adenovirus is used as an expression vector, a DETH coding sequence may be ligated into an adenovirus transcription/translation complex consisting of the late promoter and tripartite leader sequence. Insertion in a nonessential E1 or E3 region of the viral genome will result in a viable virus capable of expressing DETH in infected host. In addition,
30 transcription enhancers, such as the sarcoma virus (RSV) enhancer, may be used to increase expression in mammalian host cells.

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Specific initiation signals may also be required for efficient translation of a DETH sequence. These signals include the ATG initiation codon and adjacent sequences. In cases where DETH, its initiation codon and upstream sequences are inserted into the appropriate expression vector, no additional translational control signals may be needed. However, in cases where only coding sequence, or a portion thereof, is inserted, exogenous transcriptional control signals including the ATG initiation codon must be provided. Furthermore, the initiation codon must be in the correct reading frame to ensure transcription of the entire insert. Exogenous transcriptional elements and initiation codons can be of various origins, both natural and synthetic. The efficiency of expression may be enhanced by the inclusion of enhancers appropriate to cell system in use.

In addition, a host cell strain may be chosen for its ability to modulate the expression of the inserted sequences or to process the expressed protein in the desired fashion. Such modifications of the polypeptide include, but are not limited to, acetylation, carboxylation, glycosylation, phosphorylation, lipidation and acylation. Post-translational processing which cleaves a "prepro" form of the protein may also be important for correct insertion, folding and/or function. Different host cells such as CHO, HeLa, MDCK, 293, W138, etc. have specific cellular machinery and characteristic mechanisms for such post-translation activities and may be chosen to ensure the correct modification and processing of the introduced, foreign protein.

For long-term, high-yield production of recombinant proteins, stable expression is preferred. For example, cell lines which stably express DETH may be transformed using expression vectors which contain viral origins of replication or endogenous expression elements and a selectable marker gene. Following the introduction of the vector, cells may be allowed to grow for 1-2 days in an enriched media before they are switched to selective media. The purpose of the selectable marker is to confer resistance to selection, and its presence allows growth and recovery of cells which successfully express the introduced sequences. Resistant clumps of stably transformed cells can be proliferated using tissue culture techniques appropriate to the cell type.

Any number of selection of systems may be used to recover transformed cell lines. These include, but are not limited to, the herpes simplex virus thymidine kinase (Wigler M et al (1977) Cell 11:233-32) and adenine phosphoribosyltransferase (Lowy I et al (1980) Cell

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22:817-23) genes which can be employed in TK- or apt cells, respectively, Also, antimetabolite, antibiotic or herbicide resistance can be used as the basis for selection; for example, dhfr which confers resistance to methotrexate (Wigler M et al 1977) Proc Natl Acad Sci 77:3567-70); apt, which confers resistance to the Acad Sci 77:3567-70); apt, which
5 confers resistance to the aminoglycosides neomycin and G-418 */cikbere0Garapin F et al (1981') J Mol Biol. 150:1-14) and als or pat, which confer resistance to chlorsulfuron and phosphiaotricin acetyltransferase, respectively (Murry, supra). Additional selectable genes which have been descried for ample, trpB which allows cells to utilize indole in place of tryptophan, or hisD, which allows cells to utilize histinol in place of histidine ()Hartman SC
10 and RC Mulligan (1988) Proc Natl Acad Sci 85:8047-51). Visible markers such as anthocyanins, β -glucuronidase and its substrate, GUS, and luciferase and its substrate, luciferin, can be used not only to identify transformants, but also to quantify the amount of transient or stable protein expression attributable to a specific vector system (Rhodes C A et al (1995) Methods Mol Biol 55:121-131).

15 Although the presence/absence of marker gene expression suggests that the gene of interest is also present, its presence and expression should be confirmed. For example, if the sequence employing DETH is inserted within a marker gene sequence, recombinant cells containing DETH can be identified by the absence of marker gene function. Alternatively, a marker gene in response to induction or selection usually indicates expression of the tandem
20 DETH as well.

Alternatively, host cells which contain the coding sequence for DETH and express DETH may be identified by a variety of procedures known to those of skill in the art. These procedures include, but are not limited to, DNA-DNA or DNA-RNA hybridization an protein bioassay or immunoassay techniques which include membrane, solution, or chip based
25 technologies for the detection and/or quantification of the nucleic acid or protein.

The presence of the DETH polynucleotide sequence can be detected by DNA-DNA or DNA-RNA hybridization or amplification using probes, portions or fragments of DETH. Nucleic acid amplification based assays involve the use of oligonucleotides or oligomers based on the DETH sequence to detect transformants containing DETH DNA or RNA. As
30 used herein "oligonucleotides" or "oligomers" refer to a nucleic acid sequence of at least about

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10 nucleotides and as many as about 60 nucleotides, preferably about 15 to 30 nucleotides, and more preferably about 20-25 nucleotides which can be used as a probe or amplimer.

A variety of protocols that can be used for detecting and measuring the expression of DETH, using either polyclonal or monoclonal antibodies specific for the protein are known in the art. Examples include enzyme-linked and immunosorbent assay (ELISA),
5 radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site, monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfacing epitopes on DETH is preferred, but a competitive binding assay may be employed. These and other ways are described, among other places, in Hampton R et al (1990,
10 *Serological Methods, a Laboratory Manual*, APS Press, St. Paul Minn.) and Maddox C E et al (1983, J. Exp. Med. 158:1211).

A wide variety of labels and conjugation techniques are known by those skilled in the art and can be used in various nucleic acid and amino acid assays. Means for producing labeled hybridization or PCR probes for detecting sequences related to DETH include
15 oligolabeling, nick translation, endlabelling or PCR amplification using a labeled nucleotide. Alternatively, the DETH sequence, or any portion of it, may be cloned into a vector for the production of an mRNA probe. Such vectors are known in the art, are commercially available, and may be used to synthesize RNA probes in vitro by addition of an appropriate RNA polymerase such as T7, T3 or SP6 and labeled nucleotides.

20 A number of companies such as Pharmacia Biotech (Piscataway NJ.), Promega (Madison Wis.), and US Biochemical Corp (Cleveland Ohio) supply commercial kits and protocols for these procedures. Suitable reporter molecules or labels include those radionuclides, enzymes, fluorescent, chemiluminescent, or chromogenic agents as well as substrates, cofactors, inhibitors, and magnetic particles and the like. Patents teaching the use
25 of such labels include U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149 and 4,366,241. Also, recombinant immunoglobulins may be produced as shown in U.S. Pat. No. 4,816,567 incorporated herein by reference.

Host cells transformed with DETH nucleotide sequence may be cultured under conditions suitable for the expression and recovery of the encoded protein from cell culture.
30 The protein produced by a recombinant cell may be secreted or contained intracellularly depending on the sequence and/or the vector used. As will be understood by those of skill in

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the art, expression vectors containing DETH can be designed with signal sequences which direct secretion of DETH through a prokaryotic or eukaryotic cell membrane. Other recombinant constructions may join DETH to nucleotide sequence encoding a polypeptide domain which will facilitate purification of soluble proteins (Kroll D J et al (1993) DNA Cell Biol 12:441-53; of discussion of vectors infra containing fusion proteins).

DETH may also be expressed as recombinant protein with one or more additional polypeptide domains added to facilitate protein purification. Such purification facilitating domains include, but are not limited to, metal chelating peptides such as histidine-tryptophan modules that allow purification on immobilized metals, protein A domains that allow purification on immobilized immunoglobulin, and the domain utilized in the FLAGS extension/affinity purification system (Immunex Corp, Seattle Wash.). The inclusion of a cleavable linker sequences such as Factor XA or enterokinase (Invitrogen, San Diego Calif.) between the purification domain and DETH is useful to facilitate purification. One such expression vector provides for expression of a fusion protein comprising an DETH and contains nucleic acid encoding 6 histidine residues followed by thioredoxin and an enterokinase cleavage site. The histidine residues facilitate purification on IMIAC (immobilized metal ion affinity chromatography as described in Porath et al (1992) Protein Expression and Purification 3:23-281) while the enterokinase cleavage site provides a means of purifying the chemokine from the fusion protein.

In addition to recombinant production, fragments of DETH may be produced by direct peptide synthesis using solid-phase techniques (cf Stewart et al (1969) *Solid-Phase Peptide Synthesis*, W H Freeman Co, San Francisco; Merrifield synthesis may be performed using manual techniques or by automation. Automated synthesis may be achieved, for example, using Applied Biosystems 431A Peptide Synthesizer (Perkin Elmer, Foster City Calif.) in accordance with the instructions provided by the manufacturer. Various fragments of DETH may be chemically synthesized separately and combined using chemical methods to produce the full length molecule.

DETH-specific antibodies are useful of the diagnosis of conditions and diseases associated with expression of DETH. DETH for antibody induction does not require biological activity; however, the protein fragment, or oligopeptide must be antigenic. Peptides used to induce specific antibodies may have an amino acid sequence consisting of at

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least five amino acids, preferably at least 10 amino acids. They should mimic a portion of the amino acid sequence of the natural protein and may contain the entire amino acid sequence of a small, naturally occurring molecule. Short stretches of DETH amino acids may be fused with those of another protein such as a keyhole limpet hemocyanin and antibody produced against the chimeric molecule. Procedures well known in the art can be used for the production of antibodies to DETH. Such antibodies include, but are not limited to, polyclonal, monoclonal, chimeric, single chain. Fab fragments and fragments produced by a Fab expression library. Neutralizing antibodies, i.e., those which inhibit dimer formation, are especially preferred for diagnostics and therapeutics.

For the production of antibodies, various hosts including goats, rabbits, rats, mice, etc. may be immunized by injection with DETH or any portion, fragment or oligopeptide which retains immunogenic properties. Depending on the host species, various adjuvants may be used to increase immunological response. Such adjuvants include but are not limited to Freund's, mineral gels such as aluminum hydroxide, and surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, keyhole limpet hemocyanin, and dinitrophenol. BCG (bacilli Calmette-Guerin) and *Corynebacterium parvum* are potentially useful human adjuvants.

Monoclonal antibodies to DETH may be prepared using any technique which provides for the production of antibody molecules by continuous cell lines in culture. These include but are not limited to the hybridoma technique originally described by Koehler and Milstein (1975 Nature 256:495-497), the human B-cell hybridoma technique (Koshor et al (1983) Immunol Today 4:72; Cote et al (1983) Proc Natl Acad Sci 80:2026-2030) and the EBV-hybridoma technique (Cole et al (1985) *Monoclonal Antibodies and Cancer Therapy*, Alan R Liss Inc, New York N.Y., pp 77-96).

In addition, techniques developed for the production of "chimeric antibodies", the splicing of mouse antibody genes to human antibody genes to obtain a molecule with appropriate antigen specificity and biological activity can be used (Morrison et al (1984) Proc Natl Acad Sci 81:6851-6855; Neuberger et al (1984) Nature 312:604-608; Takeda et al (1985) Nature 314:452-454). Alternatively, techniques described for the production of single chain antibodies (U.S. Pat. No. 4,946,778) can be adapted to produce DETH specific single chain antibodies.

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Antibodies may also be produced by inducing *in vivo* production in the lymphocyte population or by screening recombinant immunoglobulin libraries or panels of highly specific binding reagents as disclosed in Orlandi et al (1989, Proc Natl Acad Sci 86:3833-3837), and Winter G and Milstein C (1991; Nature 349:293-299).

5 Antibody fragments which contain specific binding sites for DETH may also be generated. For example, such fragments include, but are not limited to, the F(ab')₂ fragments. Alternatively, Fab expression libraries may be constructed to allow rapid and easy identification of monoclonal Fab fragments with the desired specificity (Huse W D et al (1989) Science 256:1275-1281).

10 DETH antibodies are useful in the diagnosis of conditions or diseases in which the expression of DETH is characteristic of the condition or disease, or in assays to monitor patients being treated with DETH, an agonist of DETH or an inhibitor of DETH. Diagnostic assays for DETH include methods utilizing the antibody and a label to detect DETH in human body fluids or extracts of cells or tissues. The polypeptides and antibodies of the present
15 invention may be used with or without modification. Frequently, the polypeptides and antibodies will be labeled by joining them, either covalently or noncovalently, with a reporter molecule. A wide variety of reporter molecules are known as described herein and known in the art.

 A variety of protocols for measuring DETH or fragment or variant thereof, using
20 either polyclonal or monoclonal antibodies specific for the respective protein are known in the art. Examples include enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA) and fluorescent activated cell sorting (FACS). A two-site monoclonal-based immunoassay utilizing monoclonal antibodies reactive to two non-interfering epitopes on DETH can be used as can a competitive binding assay. These assays and other immunoassays
25 are well known in the art and can be found in standard texts such as Antibodies, A Laboratory Manual, Harlow and Lane, editors, Cold Spring Harbor Laboratory, Cold Spring Harbor, New York (1988).

 In order to provide a basis for diagnosis, normal or standard values for DETH expression must be established. This may be accomplished, for example, by testing body
30 fluids or cell extracts taken from normal subjects, either animal or human, in immunoassays such as those described herein, to establish a baseline level of DETH. Standard values

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obtained from normal samples may then be compared with values obtained from samples from subjects known to be affected or potentially affected by a disease or condition where apoptosis is implicated or where levels of DETH are associated with the presence or absence of. The difference between the baseline levels of DETH and the levels in a subject known or potentially affected by disease establishes or assists in establishing the presence of disease state.

The present invention provides methods for detecting the presence of DETH or nucleic acid encoding DETH in a tissue or other sample. For example, a detectably labeled antibody specific for DETH, variant or fragment thereof is added to a test sample of cells, biological tissues or fluids under conditions that allow bonding to DETH in the cells, tissue or fluid. Binding of labeled antibody is then measured. The presence of detectable label in the sample indicates the presence of DETH. Similarly, the presence of nucleic acid encoding DETH, variant or fragment thereof can be detected by adding detectably labeled nucleic acid sequence encoding DETH, variant or fragment thereof or a corresponding non-coding sequence to a test sample of cells biological tissue or fluid under conditions that allow hybridization of the labeled nucleic acid to nucleic acid encoding DETH. Hybridization of the labeled nucleic acid is then measured. The presence of detectable label in the sample indicates the presence in the sample of nucleic acid encoding DETH.

For use as a reagent in such assays, DETH, variant or fragment thereof, nucleic acid encoding encoding DETH, variant or fragment thereof or antibody specific for DETH preferably incorporates a detectable label, thus providing an additional embodiment of the invention. The detectable label can be any conventional type of label and is selected in accordance with the type of assay to be performed. For example, the detectable label can comprise a radiolabel such as ^{14}C , ^{125}I , or ^3H , an enzyme such as peroxidase, alkaline or acid phosphatase, a fluorescent label such as fluorescein isothiocyanate (FITC) or rhodamine, an antibody, an antigen, a small molecule such as biotin, a paramagnetic ion, a latex particle, an electron dense particle such as ferritin or a light scattering particle such as colloidal gold. Suitable methods to detect such labels include scintillation counting, autoradiography, fluorescence measurement, calorimetric measurement or light emission measurement. Suitable assays and procedures for accomplishing such labeling and detection of the labels are well known in the art and can be found, for example, in An Introduction to

Radioimmunoassays and Related Techniques: Laboratory Techniques in Biochemistry and Molecular Biology, 4th Ed., T. Chard, Elsevier Science Publishers, Amsterdam, The Netherlands, 1990; Methods in Non-Radioactive Detection, Gary C. Howard, Ed., Appleton and Lange, East Norwalk, Ct, 1993 or Radioisotopes in Biology: A Practical Approach, R.J. Slater, Ed., IRL Press at Oxford University Press, Oxford, England, 1990.

For example, an antibody specific for DETH can be labeled with ^{125}I according to conventional methods known in the art and used to determine the location of DETH in a tissue sample such as a sample of brain tissue or stomach tissue. The labeled antibody will bind to DETH in the tissue and the presence of the ^{125}I label can be detected by scintillation counting or autoradiography, thus signaling the presence in the tissue of DETH.

In those disorders where the induction of apoptosis is desirable, DETH or fragment thereof, could be used as a therapeutic to trigger apoptosis. A nucleic acid sequence encoding DETH, variant or fragment thereof, could be targeted to and expressed within a cell type to induce apoptosis. Alternatively, DETH or fragment thereof could be delivered to particular cells through a number of targeting techniques. Apoptosis is desirable in autoimmune disorders such as rheumatoid arthritis, multiple sclerosis and myasthenia gravis and cancers such as lymphomas, intestinal and breast cancers.

Another embodiment of the present invention provides methods of identifying inhibitors or inducers of apoptosis, which compounds can then be used as therapeutics.

DETH or fragment thereof or variant thereof can be used to screen for compounds such as small organic molecules, peptides and other types of molecules that induce apoptosis, which compounds can then be used as therapeutics to induce apoptosis. For example, a test compound can be added to cells expressing low levels of DETH or fragment thereof and control cells. Compounds that induce the death of only the DETH-expressing cells would thus be inducers of apoptosis. Such compounds could then be used in the treatment of disorders where apoptosis is desirable, such as autoimmune disorders such as rheumatoid arthritis, multiple sclerosis, myasthenia gravis and cancers such as lymphomas, intestinal and breast cancer. Thus, the present invention provides a method for identifying compounds that induce apoptosis comprising the steps of adding a test compound to cells transformed to express low levels of DETH, variant, or fragment thereof and determining whether the cells live or die. If the test compound induces cell death by apoptosis, it can be used as a

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therapeutic as discussed above. The morphological changes in cells which are associated with apoptosis are well known and can be used to determine the status of the cells.

In those disorders where apoptosis is not desirable, such as stroke, Alzheimer's disease ALS, spinal muscular atrophy, Huntington's Chorea and Parkinson's Disease, an inhibitor of DETH could be used as a therapeutic to inhibit apoptosis. Inhibitors of DETH can be obtained through screening mammalian cells that transiently over express DETH, variant or fragment thereof. A test compound is then added to such cells whereby when the test compound inhibits apoptosis, the cells survive. Thus, the present invention provides a method for identifying compounds that inhibit apoptosis comprising adding a test compound to cells transformed to overexpress DETH, fragment or variant thereof and determining whether the cells live or die. If the test compound inhibits apoptosis, the cells will live. If the compound is not effective in inhibiting apoptosis, the cells will die.

The DETH protein can be used to identify cellular or other types of ligands that bind to the protein. For example, DETH or fragment thereof can be used to make protein affinity columns to trap proteins or other types of ligands present in cell lysate. The proteins eluting from the affinity column can be analyzed to identify the protein or other ligand and its function.

This invention also contemplates the use of competitive drug screening assays in which neutralizing antibodies capable of binding DETH specifically compete with a test compound for binding DETH. In this manner, the antibodies can be used to detect the presence of any peptide which shares one or more antigenic determinants with DETH.

A polynucleotide, DETH, or any part thereof, may be used for diagnostic and/or therapeutic purposes. For diagnostic purposes, the DETH of this invention may be used to detect and quantitate gene expression in biopsied tissues in which expression of DETH may be implicated. The diagnostic assay is useful to distinguish between absence, presence, and excess expression of DETH and to monitor regulation of DETH levels during therapeutic intervention. Included in the scope of the invention are oligonucleotide sequences, antisense RNA and DNA molecules, and PNAs.

The present invention provides pharmaceutical compositions comprising nucleic acid sequences, proteins, antibodies, agonists, or inhibitors, alone or in combination with at least

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one other agent, such as stabilizing compound, and a pharmaceutically acceptable carrier or diluent. Suitable carriers and diluents include saline, buffered saline, dextrose, and water. Any of these molecules can be administered to a patient alone, or in combination with other agents, drugs or hormones, in pharmaceutical compositions where it is mixed with
5 excipient(s) or pharmaceutically acceptable carriers. In one embodiment of the present invention, the pharmaceutically acceptable carrier is pharmaceutically inert.

Administration of pharmaceutical compositions is accomplished by any suitable route, such as orally or parenterally. Methods of parenteral delivery include topical, intra-arterial (directly to the tumor), intramuscular, subcutaneous, intramedullary, intrathecal,
10 intraventricular, intravenous, intraperitoneal, or intranasal administration. Further details on techniques for formulation and administration may be found in the latest edition of "Remington's Pharmaceutical Sciences" (Maack Publishing Co., Exton Pa.))

Pharmaceutical compositions suitable for use in the present invention include compositions wherein the active ingredients are contained in an effective amount to achieve
15 the intended purpose. The determination of an effective dose is well within the capability of those skilled in the art.

For any compound, the therapeutically effective dose can be estimated initially either in cell culture assays, e.g., of neoplastic cells, or in animal models, usually mice, rabbits, dogs or pigs. The animal model is also used to achieve desirable concentration range and route of
20 administration. Such information can then be used to determine useful doses and routes for administration in humans.

A therapeutically effective dose refers to that amount of protein or its antibodies, antagonists, or inhibitors which ameliorate the symptoms or condition. Therapeutical efficacy and toxicity of such compounds can be determined by standard pharmaceutical procedures in
25 cell cultures or experimental animals, e.g. ED50 (the dose therapeutically effective in 50% of the population) and LD50 (the dose lethal to 50% of the population). The dose ratio between therapeutic and toxic effects is the therapeutic index, and it can be expressed as the ratio, ED50/LD50. Pharmaceutical compositions which exhibit large therapeutic indices are preferred. The data obtained from cell culture assays and animal studies is used in
30 formulating a range of dosage for human use. The dosage of such compounds lies preferably within a range of circulating concentrations that include the ED50 with little or no toxicity.

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The dosage varies within this range depending upon the dosage form employed, sensitivity of the patient, and the route of administration.

The exact dosage is chosen by the individual physician in view of the patient to be treated. Dosage and administration are adjusted to provide sufficient levels of the active moiety or to maintain the desired effect. Additional factors which may be taken into account include the severity of the disease state, e.g., tumor size and location; age, weight and gender of the patient; diet, time and frequency of administration, drug combination(s), reaction sensitivities, and tolerance/response to therapy. Long acting pharmaceutical compositions might be administered every 3 to 4 days, every week, or once every two weeks depending on half-life and clearance rate of the particular formulation.

Normal dosage amounts may vary from 0.1 to 100,000 micrograms, up to a total dose of about 1 g, depending upon the route of administration. Guidance as to particular dosages and methods of delivery is provided in the literature.

Another aspect of the subject invention is to provide for hybridization or PCR probes which are capable of detecting polynucleotide sequences, including genomic sequences, encoding DETH or closely related molecules. The specificity of the probe, whether it is made from a highly specific region, e.g., 10 unique nucleotides in the 5' regulatory region, or a less specific region, e.g. especially in the 3' region, and the stringency of the hybridization or amplification (maximal, high, intermediate or low) will determine whether the probe identifies only naturally occurring DETH, alleles or related sequences.

It will be appreciated by those skilled in the art that as a result of the degeneracy of the genetic code, a multitude of DETH-encoding nucleotide sequences, some bearing minimal homology to the nucleotide sequences of any known and naturally occurring gene may be produced. The invention has specifically contemplated each and every possible variation of nucleotide sequence that could be made by selecting combinations based on possible codon choices. These combinations are made in accordance with the standard triplet genetic code is applied to the nucleotide sequence of naturally occurring DETH, and all such variations are to be considered as being specifically disclosed.

Probes may also be used for the detection of related inhibitor encoding sequences and should preferably contain at least 50% of the nucleotides from any of the DETH encoding sequences. The hybridization probes of the subject invention may be derived from the

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nucleotide sequence of SEQ. ID NO:1 or from genomic sequence including promoter, enhancer elements and introns of the naturally occurring DETH. Hybridization probes may be labeled by a variety of reporter groups, including radionuclides such as ^{32}P or ^{35}S , or enzymatic labels such as alkaline phosphatase coupled to the probe via avidin/biotin coupling systems, and the like.

Other means for producing specific hybridization probes for DETH DNAs include the cloning of nucleic acid sequences encoding DETH or DETH derivatives into vectors for the production of mRNA probes. Such vectors are known in the art and are commercially available and may be used to synthesize RNA probes in vitro by means of the addition of the appropriate RNA polymerase as T7 or SP6 RNA polymerase and the appropriate radioactively labeled nucleotides.

Nucleic acid sequences encoding DETH may be used for the diagnosis of conditions or diseases with which the expression of DETH is associated. For example, polynucleotide sequences encoding DETH may be used in hybridization or PCR assays of fluids or tissues from biopsies to detect DETH expression. The form of such qualitative or quantitative methods may include Southern or northern analysis, dot blot or other membrane-based technologies; PCR technologies; dip stick, pin, chip and ELISA technologies. All of these techniques are well known in the art and are the basis of many commercially available diagnostic kits.

EXAMPLES

Example 1 - DNA encoding DETH

I. The novel protein DETH was identified after the assembly of expressed sequence tags (EST) sequences from a proprietary database base (Lifeseq data base, Incyte, Palo Alto, California) and the public Merck /Washington University EST sequence database. The initial identification of these ESTs was performed by basic local alignment search tool (BLAST) analysis of the databases using the death domain sequence of RIP (TNF receptor interacting protein) :

LNPIRENLGRQWKNCARKLGFTESQIDEIDHD-
YERDGLKEKVYQMLQKWLMREGTKGATVGKLAQAL

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One complete reading frame was identified which consisted of 116 EST's and covered 2715 bases. Translation of the contiguous sequence generated a putative protein sequence of 405 amino acids starting with a methionine. The putative protein contained three significant features: a transmembrane domain (amino acids 103-118), a death domain (amino acids 168-240) and a serine-proline rich region (amino acids 262-325).

Clone g1727750 which contains the 5' sequence of DETH was obtained from the Merck/ Washington University EST Project, St. Louis, Missouri, USA and sequenced using an Applied Biosystems Model 377 Automated DNA Sequencer and standard fluorescent dye terminator chemistry. The cDNA clone g1727750 was made from mRNA of a human colon epithelial cell line cloned into a vector (Unizap, Stratagene, La Jolla, California, USA) at the EcoRI and XhoI sites and was in a 2.0 ml *E. coli* culture. The sequence of clone g1727750 is shown in SEQ ID NO: 1.

A pair of primers were designed to contain 5' plus-strand sequence starting with the ATG (start codon) (Primer 1) and 3' minus-strand sequence starting at the TAG (stop codon) (Primer 2) with Bgl II and Sal I restriction sites at the 5' and 3' ends, respectively. The primers were as follows:

Primer 1 - ttt aga tct atg aac tca aca gaa tcc aac tct tct gcc

Primer 2 - gtc gac cta cag cag gtc agg aag atg gct ata aac ag

The full length coding region (SEQ ID NO: 1 bases 190-1404) was then amplified by PCR with these primers and high-fidelity Vent DNA polymerase (New England Biolabs, Inc., Beverly, Massachusetts, USA) using clone g1727750 plasmid DNA as the template in a 12 cycle PCR protocol (94°C for one minute for denaturation, 62°C for 30 seconds for annealing, 72°C for 2 minutes for extension).

The 1.26 Kb PCR product was cut with Bgl II and Sal I, phenol-precipitated and ligated into plasmid pGEX6p-1 (Pharmacia Biotech Inc., Piscataway, New Jersey USA at the Bam HI and Sal I sites. The resulting plasmid containing the 1.26 Kb PCR product was transformed into *E. coli* BL-21 (DE3)pLYSs cells (Stragagene, La Jolla, California, USA) and expressed as described in Example 4.

The PCR product was then sequenced using an Applied Biosystems Model 377 Automated DNA Sequencer and standard fluorescent dye terminator chemistry.

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Example 2 - Cloning of the extracellular and intracellular regions of DETH

The extracellular region of DETH is comprised of amino acids 1-102, coded for by the 5' 306 base pair (bp) portion of the DETH DNA sequence (SEQ ID NO: 1, bases 190-495).

The intracellular region of DETH is comprised of amino acids 121-405 coded for by 855 base pairs of the DETH open reading frame (SEQ ID NO: 1, bases, bases 550-1404).

The extracellular and intracellular regions of the DETH protein were separately cloned using the PCR protocol described in Example 1 and the primers described below.

For the extracellular region the following primers containing the restriction sites Bgl II/Sal I were used: 5' Bgl II for Primer 1 - ttt aga tct atg aac tca aca gaa tcc aac tct tct gcc

3' EcoR I for Primer 3 - ttt gaa ttc caa atg ctc att gat gtc aaa atg c

For the intracellular region the following primers containing Bgl II/ Eco RI restriction sites were used: 5' Bgl II for Primer 4 - ttt aga tct cgg aaa agc tcg agg act ctg aaa aag ggg

3' Sal I for Primer 2 - gtc gac cta cag cag gtc agg aag atg gct ata aac ag

The PCR product for the extracellular region was cut with Bgl II and Sal I and ligated into pGEX6p-1 at the Bam HI/Sal I site. The PCR product for the intracellular region was cut with Bgl II and Eco RI and ligated into pGEX6p-1 at the Bam HI/Eco RI site. The vector pGEX6p-1 (Pharmacia Biotech Inc., Piscataway, New Jersey) creates a glutathione-S-transferase (GST) fusion protein which contains a glutathione recognition site.

The respective plasmids containing the extracellular region or the intracellular region were then amplified in accordance using the protocol described in Example 4 and sequenced.

Example 3 - Expression of DETH in Human Cells

This DNA was ligated into pIRES-EGFP (Clontech, Palo Alto, California, USA) at the EcoR V site. Because the insert can be ligated in two (opposite) orientations, PCR was used to screen for the correct directionality. The recombinant DNA was transformed into E. coli and colonies were screened by a PCR method using a pair of primers, one of which was vector-specific and one that was DETH-specific. The size of the PCR product determined which plasmids contained correctly oriented inserts. Ten colonies were found carrying

-26-

plasmids with the correct orientation of the full-length DETH sequence in pIRES-EGFP. Two of the ten plasmids were sequenced and found to be error free.

HeLa cells (American Type Culture Collection (ATCC) Accession number CCL-2, Rockville, Maryland, USA) grown in Dulbecco's Minimum Essential Medium (DMEM) supplemented with 10% fetal calf serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin. A 549 cells (ATCC Accession Number CCL-185), a human lung carcinoma cell line, were grown in F-12K medium supplemented with 10% fetal calf serum, 100 U/ml penicillin and 0.1 mg/ml streptomycin. Cells were transfected by electroporation using 5-10 µg of plasmid DNA in 250 µL DMEM plus fetal calf serum and a Bio-Rad Gene Pulser II at the settings 0.22 Kr, 0.96 µF, 40-50 microseconds.

He La or A549 cells were split 24 hours prior to transfection. 2×10^6 cells were transfected with pIRES containing the full length 1.2 Kb PCR product coding for DETH. 2×10^6 cells were transfected with the pIRES vector alone and used as a negative control.

After cells were electroporated with equal amounts of DNA, they were seeded into a six-well plate with a cover slip in each well. At least 24 or 48 hours, the cover slips were picked and cells on them were fixed with 4% paraformaldehyde. With or without staining with the DNA stain 4,6-diamidino-2-phenylindole (DAPI) the coverslips were mounted on slides and examined by fluorescence microscopy. The percentage of green (fluorescent) cells from experimental samples were normalized according to the pIRES negative control. The cell survival rates were the summary of three independent experiments.

When DETH was overexpressed in He La cells, approximately 95% of cells containing the protein died by apoptosis. Apoptosis was verified by cell morphology. By contrast, there was nearly 100% survival of cells transfected with the pIRES vector only.

4. Expression of DETH and Regions of DETH as GST fusion proteins and Purification thereof

The pGEX6p-1 plasmid constructs described in Examples 1 and 2, containing DNA coding for full-length DETH, the extracellular region, or the intracellular region, respectively, were transformed into *E. coli* BL-21 (DE3)pLysS cells (Stragagene, La Jolla, California, USA). Cells carrying these plasmids were grown in LB-AMP medium (Gibco BRL, Gaithersburg, Maryland, USA) to a density of $A=0.5$ as measured on a spectrophotometer at

-27-

600 nm . Isopropyl β -D-thiogalactoside (IPTG) was added to the cell cultures to a final concentration of 0.4 mM and the cells were incubated for two more hours. The cells were then harvested and resuspended in one tenth the culture volume of phosphate buffered saline (PBS) containing 100 μ M/ml lysozyme and incubated on ice for thirty minutes to lyse the cells. The lysates were sonicated for one minute in the presence of 1% Nonidet p-40 (NP-40) and 1.0 mM phenylmethylsulfonylfluoride (PMSF) and centrifuged at 15,000 x g for forty minutes. The supernatants were poured over a 1 ml glutathione-Sepharose 4B column (Pharmacia) which was then washed with PBS and PreScission Cleavage Buffer (50 mM Tris-HCl pH 7.0, 150 mM NaCl, 1 mM EDTA, 1mM dithiothreitol, Pharmacia). The DETH protein was cleaved from GST by a protease (Precision Protease, Pharmacia), 80 U/ml). The cleaved DETH proteins were checked by SDS-PAGE for purity.

The full-length GST-DETH fusion protein has been expressed in *E. coli* but was found to be insoluble. The cytoplasmic and extracellular regions of DETH were purified to approximately 99% purity.

In addition to the above purification procedure, an ion-exchange step using a Mono-S column (Pharmacia) with a linear 50 - 500 mM NaCl gradient has been successfully used to further purify the extracellular region.

Example 5. Polyclonal antibodies

Soluble purified protein (recombinantly produced extracellular region or cytoplasmic region) were used to inject rabbits in accordance with the method in *Antibody, A Laboratory Manual* , Harlow and Lane, editors, Cold Spring Harbor Press, Cold Spring Harbor, New York, 1988.

Example 6 - Northern Blot Analysis of Normal Human Tissues and Human Cancer Cell Lines

The 1.26 kb PCR product corresponding to the coding sequence of DETH was used to make a probe for northern blots. The PCR product was labeled using a random, hexanucleotide priming kit (Boehringer Mannheim, Indianapolis, Indiana, USA) and gamma-P³²-dCTP (3,000 Ci/millimole, Amersham, Arlington Heights, Illinois, USA). The procedure was carried out according to the manufacturer's instructions.

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Northern analysis was done using a kit (Clontech Master Northern Blot, Clontech, Palo Alto, California, USA). Multiple tissue blots and tumor cell line blots used in the northern analysis were also purchased from Clontech. Northern analysis was performed in accordance with the manufacturer's instruction with the following modifications.

- 5 Hybridizations were carried out at 68°C for 2 hours in Expresshyb solution (Clontech). The blots were washed once each with two-fold concentrated SSPE buffer (Gibco/BRL, Gaithersburg, Maryland, USA), normal SSPE buffer and two-fold diluted SSPE buffer at 65°C for 30 minutes. After the blots were dried, they were exposed to Kodak XAR 5 film with an intensifying screen at -80°C for 24 hours. Alternatively, the blot membranes were
- 10 quantified by use of a phosphoimager (Storm, phosphoImager, Molecular Dynamics) with an exposure of 2 hours. This gave a relative intensity for each tissue or cell line.

The major message size found on this blot for DETH was approximately 4.0 Kb. The intensity of the signals are represented semi-quantitatively from absent (-) to very strong (+++).

15

Cancer Cell Lines	Relative Intensity
HL-60, promyelocytic leukemia	-
Hela S3, cervical carcinoma	+/-
K-562, chronic myelogenous leukemia	-
20 MOLT-4, lymphoblastic leukemia	+/-
Raji, Burkitt's lymphoma	-
SW480, colorectal adenocarcinoma	+
A549, lung carcinoma	+++
G361, melanoma	+

25

The results show that the DETH message is strongly expressed in the lung cancer cell line A549 (more than 20-fold stronger than any other cell line), and more weakly expressed in melanoma and colorectal cancer cell lines.

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	Tissue	Relative Intensity	Tissue	Relative Intensity
	whole brain	+/-	amygdala	+
	caudate nucleus	+	cerebellum	+
	cerebral cortex	++	frontal lobe	++
5	hippocampus	++	medulla oblongata	+++
	occipital lobe	-	putamen	+/-
	substantia nigra	++	temporal lobe	++
	thalamus	++++	subthalamic nucleus	+++
	spinal cord	++++	heart	+/-
10	aorta	-	skeletal muscle	-
	colon	+	bladder	++
	uterus	++	prostate	++
	stomach	+++	testis	-
	ovary	+/-	pancreas	+/-
15	pituitary gland	+/-	adrenal gland	+/-
	thyroid gland	-	salivary gland	+/-
	mammary gland	++	kidney	+
	liver	-	small intestine	+/-
	spleen	-	thymus	+/-
20	peripheral leukocyte	-	lymph node	+/-
	bone marrow	-	appendix	+
	lung	+	trachea	+
	placenta	++	fetal brain	+
	fetal heart	+/-	fetal kidney	++
25	fetal liver	-	fetal spleen	+/-
	fetal thymus	+/-	fetal lung	+

These data demonstrate that while low levels of DETH mRNA are found in many tissues, DETH is primarily a brain and, to a lesser extent, a stomach transcript.

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CLAIMS:

1. A purified protein having an amino acid sequence having at least 80% identity to DETH.
2. The purified protein of claim 1 wherein said protein has an amino acid sequence having at least 90% identity to DETH.
3. The purified protein of claim 1 wherein said protein has an amino acid sequence having at least 95 % identity to DETH.
4. The purified protein of claim 1 wherein said protein has an amino acid sequence having at least 99 % identity to DETH.
5. The purified protein of claim 1 wherein said protein has the amino acid sequence of SEQ ID NO: 2.
6. A fragment of DETH that induces apoptosis.
7. A purified nucleic acid sequence encoding a protein of any one of claims 1-6.
8. An antibody specific for DETH.
9. A method of inducing apoptosis comprising the step of expressing DETH in a cell in an amount effective to induce apoptosis.

-1-

SEQUENCE LISTING

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[illegible]

INTERNATIONAL SEARCH REPORT

In tional Application No

PCT/GB 99/01793

A. CLASSIFICATION OF SUBJECT MATTER

IPC 6 C12N15/12 C07K14/705 C07K16/28 A61K38/17

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 6 C12N C07K A61K

Documentation searched other than minimum: documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	PAN G. ET AL.: "Identification and functional characterization of DR6, a novel death domain-containing TNF receptor" FEBS LETTERS, vol. 431, 1998, pages 351-356, XP002113408 the whole document	1-9
P,X	WO 99 15663 A (MILLENNIUM BIOTHERAPEUTICS INC) 1 April 1999 (1999-04-01) sequence of TRL II	1-9
P,X	EP 0 869 179 A (SMITHKLINE BEECHAM CORP) 7 October 1998 (1998-10-07) sequence of TR7	1-9
	-/--	

☒ Further documents are listed in the continuation of box C.

☒ Patent family members are listed in annex.

* Special categories of cited documents:

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

- "T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- "X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- "Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- "&" document member of the same patent family

Date of the actual completion of the international search

26 August 1999

Date of mailing of the international search report

06/09/1999

Name and mailing address of the ISA

European Patent Office, P.E. 5818 Patentlaan 2
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Fax: (+31-70) 340-3016

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Galli, I

INTERNATIONAL SEARCH REPORT

International Application No

PCT/GB 99/01793

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
P,X	WO 98 56892 A (HUMAN GENOME SCIENCES INC ;NI JIAN (US); FAN PING (US); GENTZ REIN) 17 December 1998 (1998-12-17) sequence of TR9 ---	1-9
P,X	WO 99 11790 A (ZYMOGENETICS INC) 11 March 1999 (1999-03-11) sequence of ZTNFR-6 ---	1-9
E	WO 99 31128 A (INCYTE PHARMA INC ;AU YOUNG JANICE (US); BANDMAN OLGA (US); KASER) 24 June 1999 (1999-06-24) seq. ID 4 ---	1-9
A	KITSON J ET AL: "A DEATH-DOMAIN-CONTAINING RECEPTOR THAT MEDIATES APOPTOSIS" NATURE, vol. 384, no. 6607, 28 November 1996 (1996-11-28), pages 372-375, XP002036560 ISSN: 0028-0836 abstract ---	1-9
A	CHINNAIYAN A M ET AL: "SIGNAL TRANSDUCTION BY DR3, A DEATH DOMAIN-CONTAINING RECEPTOR RELATED TO TNFR-1 AND CD95" SCIENCE, vol. 274, no. 5289, 8 November 1996 (1996-11-08), pages 990-992, XP000676685 ISSN: 0036-8075 abstract ---	1-9
A	PAN G ET AL: "AN ANTAGONIST DECOY RECEPTOR AND A DEATH DOMAIN-CONTAINING RECEPTOR FOR TRAIL" SCIENCE, vol. 277, 8 August 1997 (1997-08-08), pages 815-818, XP002065147 ISSN: 0036-8075 abstract ---	1-9
A	PAN G ET AL: "THE RECEPTOR FOR THE CYTOTOXIC LIGAND TRAIL" SCIENCE, vol. 276, 4 April 1997 (1997-04-04), pages 111-113, XP002913702 ISSN: 0036-8075 abstract -----	1-9

INTERNATIONAL SEARCH REPORT

International application No.

PCT/GB 99/ 01793

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☒ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
Remark: Although claim 9 can be understood to comprise methods in vivo and is therefore considered a method of treatment of the human/animal body, the search has been limited to the alleged effects of the compound/composition.
2. ☐ Claims Nos.:
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No

PCT/GB 99/01793

Patent document cited in search report		Publication date	Patent family member(s)		Publication date
WO 9915663	A	01-04-1999	AU	9777698 A	12-04-1999
EP 0869179	A	07-10-1998	JP	11018786 A	26-01-1999
WO 9856892	A	17-12-1998	AU	7830398 A	30-12-1998
WO 9911790	A	11-03-1999	AU	9130598 A	22-03-1999
WO 9931128	A	24-06-1999	NONE		